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CHAPTER TWENTY

Peptidomimetics for Drug Design

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1 INTRODUCTION

In the past two decades, a wide variety of naturally occurring bioactive peptides have been discovered. These peptides function as hormones, enzyme inhibitors or substrates, growth promoters or inhibitors, neurotransmitters, and immunomodulators. Most of these peptides exhibit their biological activities through binding to corresponding acceptor molecules (receptors or enzymes). Each acceptor molecule plays a unique biological role, allowing the interaction of bioactive peptides with acceptor molecules to control specific physiological events. This characteristic can allow bioactive peptides to act as therapeutic agents. Extensive studies have been undertaken in an effort to understand the physiological effects of these bioactive peptides. Unfortunately, the use of native peptides for clinical applications has been limited by intrinsic properties of peptides.

One of the most important considerations which limits the clinical application of native peptides is rapid degradation of peptides by peptidase enzymes. There are many specific and nonspecific peptidases in biological systems which rapidly metabolize peptides. Such metabolic instability complicates oral delivery of peptides. Passage through the blood–brain-barrier is an additional problem for peptides which act in the central nervous system (CNS).

Bioactive peptides act on specific acceptor molecules. However, because of the inherent flexibility of peptides, they may adopt different conformations required for recognition by multiple acceptor molecules. For example, receptors often have subtypes related to different physiological phenomena. Each subtype may require different conformations of peptides for binding. In many cases, native peptides can bind to more than one receptor subtype and this property may lead to undesirable side effects.

In an effort to counteract these detrimental properties, numerous modifications of peptide structure have been considered. These modified structures are referred to as peptidomimetics; chemical structures de-

rived from bioactive peptides which imitate natural molecules. It is widely believed that such modifications will enhance the desirable properties and avoid undesirable properties of native peptides. Many analogs incorporating peptidomimetic components have exhibited improved pharmacological and pharmacokinetic properties, including increased bioactivity, selectivity, metabolic stability, absorption, and lower toxicity. Some of these peptidomimetic analogs are currently used as therapeutic agents.

Since bioactive peptides must adopt a specific conformation to bind to an acceptor molecule, the exploration of a binding conformation is one of the most important processes involved in the effort to obtain potent and selective therapeutic agents. For this purpose, constrained peptidomimetics based on cyclic structures, constrained amino acids or amide isosteres, and mimics of peptide secondary structures have been employed. These peptidomimetics cause the resulting peptides to adopt distinct preferred conformations by removing the flexibility of the parent linear peptides. In many cases, such conformational changes are accompanied by alterations in the bioactivity profiles of the resulting analogs. Thus, the comparison of bioactivities and the effects of peptidomimetics on the overall conformations of peptides (obtained from conformational studies using modern techniques of spectroscopy and molecular modeling) can provide insight into the structures required for bioactivity. The resulting structural information can be used for the design of more effective peptidomimetics in an effort to generate the required conformations for high selectivity and potency.

To improve the metabolic stability of native peptides, amide isosteres have been incorporated into peptide bonds which are particularly susceptible to enzymatic degradation. In many cases, these modifications, as well as the incorporation of constrained peptidomimetics can enhance metabolic stability. The transformation of a peptide structure to a completely nonpeptidic molecule (retaining pharmacophores and the required three-dimensional array) is an attractive approach to the development of therapeutic agents from native peptides. These types of drug candidates can incorporate metabolic stability and oral bioavailability. In addition, these molecules, which are relatively rigid compared with peptides, can provide desirable selectivities. Other pharmacokinetic properties (solubility, hydrophobicity, transport characteristics, etc.) can also be enhanced from small changes in peptidomimetic structures.

This chapter will focus on the general features of representative peptidomimetic strategies including: cyclization of peptides, incorporations of unnatural amino acids, linkages between consecutive residues, replacements of peptide bond with amide isosteres and transformations of the secondary structure of peptides to nonpeptidic molecules. Conformational preferences, physical properties, and applications to drug design of the above peptidomimetics will be described.

2 CYCLIZATION OF PEPTIDES

2.1 General Features

Cyclization of peptides reduces the degrees of freedom for each constituent within the ring. This modification can substantially reduce the flexibility of parent linear molecules and stabilize specific secondary structures of peptides. Furthermore, if the conformation stabilized by the cyclization closely resembles the structure responsible for the bioactivity, this modification can increase potency and selectivity of the resulting peptides. Cyclic structures have been observed in many native peptides such as somatostatin, oxytocin, cyclosporin A, atrial natiuretic peptides, calcitonin and peptide antibiotics. However, the size of the cyclic rings of these peptides are too

large to generate conformationally constrained structures. Thus, smaller cyclic structures (11 to 18 membered rings) have been considered in peptidomimetic design. Cyclic structures have been incorporated into numerous bioactive peptides leading to highly potent and selective analogs.

One of the most important applications of this modification is in the studies of opioid peptides. From X-ray crystallographic studies of enkephalins (Tyr-Gly-Gly-Phe-Leu/Met-OH), two different types of structures were reported (1, 2). One of these structures adopts a β-turn stabilized by two antiparallel hydrogen bonds between Tyr^1 and Phe^4 (1). The other structure contains the fully extended conformation (2). The spectroscopic and computational studies of enkephalins support these results by showing the existence of several different conformations in equilibrium (3–5). To avoid the fully extended structure in the conformational equilibrium of enkephalins, Schiller et al. have cyclized an enkephalin analog (6).

There are four different ways to cyclize linear peptides: connection of the amino terminus to the carboxyl terminus; the amino terminus to a side chain; a side chain to the carboxyl terminus; and one side chain to another side chain. However, since the free amine of Tyr^1 is required for opioid activity, only the side chain to carboxyl terminus and the side chain to side-chain cyclizations can be used, leading to three series of analogs: Tyr-c[D-X-Gly-Phe-Leu] [X = Lys, Orn, A_2bu (diaminobutyric acid) and A_2pr (diaminopropionic acid)] (6–9), Tyr-c[D-Cys/Pen-Gly-Phe-(D/L)-Cys/Pen]-OH/NH_2 (10–12) (Pen stands for penicillamine which is β,β-dimethyl cystein) and Tyr-c(D-Lys-Gly-Phe-Glu)-OH (Fig. 20.1) (13).

The cyclic structures of the Tyr-c(D-X-Gly-Phe-Leu) series are formed through the lactam bridge between the side chain of the second residue and the carboxyl terminus of Leu (5). The resulting analogs

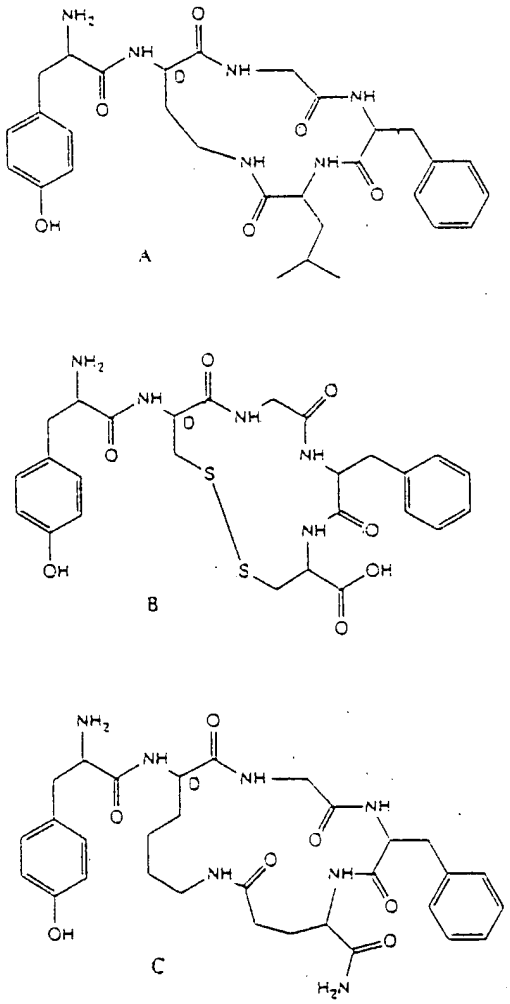


Fig. 20.1 The typical cyclic enkephalin analogs. A, Tyr-c[D-A_2bu-Gly-Phe-Leu]; B, Tyr-c[D-Cys-Gly-Phe-Cys]-OH; and C, Tyr-c[D-Lys-Gly-Phe-Glu]-NH_2.

show higher activities than enkephalins in the GPI (guinea pig ileum) assays which represent bioactivities of opioids at the μ or morphine-binding opioid receptor. On the contrary, lower activities are observed in the MVD (mouse vas deferens) tests which represent bioactivities at the δ or enkephalin-binding opioid receptor (Table 20.1) (6–9). Namely, the resulting cyclic analogs are highly μ-receptor selective. These bioactivity profiles are different from

parent enkephalins which show δ-receptor selective activities (Table 20.1). These profiles are also different from those of Tyr-Xaa-Gly-Phe-NH_2 [Xaa = D-Ala, D-Nva (norvaline) and Nle (norleucine)] which are the real parent analogs of this cyclic peptide series (9). All of these linear parent analogs are nonselective. The results indicate that the cyclization of δ-selective or nonselective enkephalins leads to μ-selective analogs. The cyclization reduces the possibility that linear enkephalins will adopt the conformations which can be recognized by the δ opioid receptor.

Another cyclic enkephalin series, Tyr-c[D-Cys/Pen-Gly-Phe-(D,L)-Cys/Pen]-OH/NH_2, has been synthesized through the disulfide bridge formation between Cys/Pen residues in positions 2 and 5 (10–12). The Cys containing analogs are superactive at both the μ- and δ-receptors and the Pen containing analogs are highly δ-receptor selective (Table 20.1) (10). These results demonstrate that β,β-dimethylation of Cys residues excludes the conformation for μ opioid receptor recognition in this cyclic peptide system. The Pen containing analogs will be discussed again later. The Tyr-c(D-Lys-Gly-Phe-Glu)-NH_2 analog was also prepared by lactam formation between the free amine of the Lys sidechain and the carboxylic acid of the Glu side chain. This analog is active at both μ- and δ-receptors (Table 20.1) (13). These results indicate that the bioactivities of analogs can be varied by means of cyclization, changes in ring sizes, and conformational constraints.

Another peptide opioid dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) (14), was also cyclized in the laboratories of Goodman (15–19), Schiller (20–22) and Spatola (23). The linear *N*-terminal tetrapeptide and pentapeptide of dermorphin retain the bioactivity of dermorphin (24). Since the size of the cyclic structure must be small in order to obtain highly constrained structures, these sequences have been employed for the cyclization of dermorphin. Among the resulting analogs,

Table 20.1 Various Cyclic Enkephalins and their Bioactivities

Analog	Cyclization and Ring Size	GPI[a] IC_{50}/nM	MVD[b] IC_{50}/nM	MVD/GPI IC_{50}-Ratio	Reference
Tyr-c(D-A_2pr-Gly-Phe-Leu)	(sm[c], 13)	23.4 ± 4.2	73.1 ± 14.5	3.12	9
Tyr-c(D-A_3bu-Gly-Phe-Leu)	(sm, 14)	14.1 ± 2.9	81.4 ± 5.8	5.77	9
Tyr-c(D-Orn-Gly-Phe-Leu)	(sm, 15)	48 ± 4.3	475 ± 99	9.90	9
Tyr-c(D-Lys-Gly-Phe-Leu)	(sm, 16)	4.80 ± 1.79	141 ± 28	29.4	9
Tyr-c(D-Cys-Gly-Phe-Cys)-OH	(ss[d], 14)	3.06	0.19	0.062	11
Tyr-c(D-Cys-Gly-Phe-D-Cys)-OH	(ss, 14)	1.48	0.12	0.081	11
Tyr-c(D-Cys-Gly-Phe-Cys)-NH_2	(ss, 14)	1.51 ± 0.03	0.760 ± 0.086	0.503	11
Tyr-c(D-Cys-Gly-Phe-D-Cys)-NH_2	(ss, 14)	0.780 ± 0.010	0.298 ± 0.037	0.382	11
Tyr-c(D-Pen-Gly-Phe-Pen)-OH	(ss, 14)	10000	2.5	0.00025	12
Tyr-c(D-Pen-Gly-Phe-D-Pen)-OH	(ss, 14)	25250	3.40	0.00014	12
Tyr-c(D-Lys-Gly-Phe-Glu)-NH_2	(ss, 18)	1.13 ± 0.14	0.648 ± 0.132	0.573	13
Tyr-Gly-Gly-Phe-Leu-OH		246 ± 39	11.4 ± 1.1	0.0463	

[a]The GPI stands for guinea pig ileum which is a muscle preparation for the *in vitro* assay to measure bioactivities at the μ opioid receptor.

[b]The MVD stands for mouse vas deferens which is a muscle preparation for the *in vitro* assay to measure bioactivities at the δ opioid receptor.

[c]The sm denotes the side chain to main chain cyclization.

[d]The ss denotes the side chain to side chain cyclization.

Table 20.2 Various Cyclic Dermorphin Analogs and their Biological Activities

Analog	Cyclization and Ring Size	GPI[a] IC_{50}/nM	MVD[b] IC_{50}/nM	MVD/GPI IC_{50}-Ratio	Reference
Tyr-c(D-Orn-Phe-Asp)-NH_2	(ss[c], 13)	36.2 ± 3.7	3880 ± 840	107	22
Tyr-c(D-Cys-Phe-Cys)-NH_2	(ss, 11)	64.7 ± 11.9	740 ± 187	11.4	22
Tyr-c(D-Orn-Phe-Gly)	(sm[d], 12)	8.60 ± 0.78	145 ± 15	16.9	15
Tyr-c(D-Lys-Phe-Ala)	(sm, 13)	0.19	0.51	2.7	23
Tyr-c(D-A$_2$bu-Phe-Ala-Leu)	(sm, 14)	0.63 ± 0.049	1.72 ± 0.27	2.71	15
Tyr-D-Ala-Phe-Gly-NH_2		45.2 ± 3.19	510 ± 31.82	11.3	24

[a]The GPI stands for guinea pig ileum which is a muscle preparation for the *in vitro* assay to measure bioactivities at the μ opioid receptor.

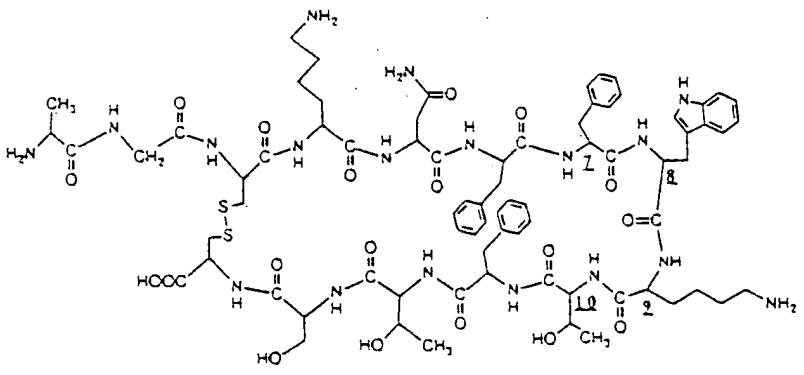
[b]The MVD stands for mouse vas deferens which is a muscle preparation for the *in vitro* assay to measure bioactivities at the δ opioid receptor.

[c]The ss denotes the side chain to main chain cyclization.

[d]The sm denotes the side chain to main chain cyclization.

Tyr-c(D-Orn-Phe-Asp)-NH_2 (20–22), Tyr-c(D-Cys-Phe-Cys)-NH_2 (22) and Tyr-c(D-Orn-Phe-Gly) (15, 17, 18) are μ-receptor selective. The analog Tyr-c[D-Orn-Phe-Asp)-NH_2 is active only at the μ-receptor. On the other hand, Tyr-c(D-Lys-Phe-Ala) and Tyr-c(D-A$_2$bu-Phe-Ala-Leu) show superactivity at both the μ- and δ-receptors (Table 20.2) (16). The cyclic dermorphin analogs with the tetrapeptide sequence are highly constrained because of the small size of the ring.

Cyclic somatostatins are another representative example of cyclic peptides. The native somatostatin contains a large cyclic structure formed by a disulfide bridge (Fig. 20.2) (25). The structure–activity relationship studies of this molecule indicate that the Phe-Trp-Lys-Thr (26–30) sequence is important for the bioactivity of somatostatin. Replacement of Trp with D-Trp increases activity (31). Furthermore, conformational analysis of somatostatin and its analogs proposed that a β-turn within the



H_2N-Ala-Gly-Cys-Lys-Asn-Phe-Phe^7-Trp^8-Lys^9-Thr^{10}-Phe-Thr-Ser-Cys-H

Fig. 20.2 Structure and sequence of native somatostatin.

Table 20.3 Growth Hormone Release Inhibition by Cyclic Somatostatin Analogs

Analog	Relative Potency[a]	Reference
D-Phe-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-ol	5	34
c(Pro-Phe-D-Trp-Lys-Thr-Phe)	1.7	27, 28
c[(NMe)-α-benzyl-O-AMPA-Phe-D-Trp-Lys-Thr](I)	<0.0001	37
c[(NMe)-α-benzyl-O-AMPA-Phe-D-Trp-Lys-Thr](II)	0.013	37

[a] A ratio between the IC_{50} value for the inhibition of growth hormone release by cyclic somatostatin analog and the IC_{50} value for the inhibitin of growth hormone release by native somatostatin.

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above sequence is required for receptor recognition (31, 32). To stabilize such a secondary structure around the pharmacophoric sequence of somatostatin, analogs with smaller ring size were synthesized. Such efforts resulted in the highly active lead analogs D-Phe-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-ol (33, 34) and c(Pro-Phe-D-Trp-Lys-Thr-Phe) (26) (Table 20.3). The former analog is synthesized by a side chain to side chain cyclization while the cyclic structure of the latter analog is formed by an amino terminus to carboxyl terminus cyclization of a hexapeptide derivative.

2.2 Constrained Units for Cyclization of Peptides

Specific functional units have been utilized to enhance the stability of specific molecular conformations. The conformational analyses of the somatostatin analogs based on the structure c(Pro^6-Phe^7-D-Trp^8-Lys^9-The^{10}-Phe^{11}) (the superscript numbers show the relative positions of amino acids with respect to native somatostatin) indicate that a cis peptide bond between Phe^{11}–Pro^6 is important for high bioactivity (26). To mimic such a cis amide bond, Van Binst et al. (35, 36) have incorporated the *o*, *m*, or *p*-aminomethylphenylacetic acids (*o*, *m*, or *p*-AMPA) into the bridging region to replace the Phe^{11}-Pro^6 dipeptide sequence. However, all of these analogs are inactive. The conformational studies of these analogs indicated that the inactivity arose from the absence of the aromatic ring corresponding to the side chain of Phe^{11} and distortions on the conformation of the pharmacophoric sequence as well as the bridging region. These conformational studies also showed that hydrogen-bonding between the NH and C(O) groups of *o*-AMPA may explain the inactivity of these analogs. To avoid this hydrogen-bonding, *N*-methylation on the amine of *o*-AMPA has been considered. Since the side chain aromatic ring of Phe^{11} was important for bioactivity, the introduction of benzyl group was also considered. Thus, *N*-methyl-α-(*R&S*)-benzyl-*o*-AMPA (37) (Fig. 20.3A) has been incorporated into the bridging region. One of the two isomers has shown substantial bioactivity (Table 20.3). A similar constrained unit, *p*-aminomethylbenzoic acid (*p*-AMBA) was incorporated into a arginyl-glycinyl-aspatyl sequence containing peptide (RGD peptide) leading to an active inhibitor of cell adhesion (Fig. 20.3B) (38). Recently, the incorporation of *o*- and *m*-aminobenzoic acid into inactivators of trypsinlike proteinases was also reported (39).

Sulfhydryl containing constrained units are useful to provide constraints and increase hydrophobicities. Derivatives of desaminocystein (Fig. 20.4) such as β,β-pentamethylene-β-mercaptopropionic acid (Ppa), 2-mercaptobenzoic acid (Mba),

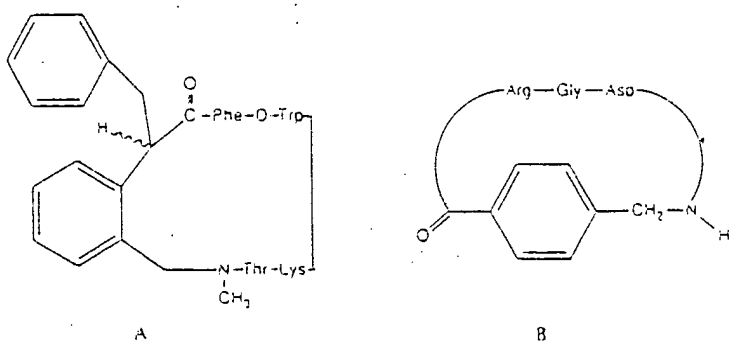


Fig. 20.3 Structures of A, a somatostatin analog c[(*N*-Methyl)-α-benzyl-o-AMPA-Phe-D-Trp-Lys-Thr]; and B, a modified RGD peptide c[Arg-Gly-Asp-pAMBA].

β,β-dimethyl-β-mercaptopropionic acid (Dmpa), and β,β-diethyl-β-mercaptopropionic acid (Depa) have also been incorporated into bioactive peptides including antagonists of vasopressin, oxytocin, RGD peptides, and others (40–44). When the Ppa, Mba, Dmpa, and Depa were incorporated into position X of a vasopressin analog [X-Phe(NO_2)-Phe-Val-Asn-Cys]-Pro-Lys-D-Tyr-NH_2, the resulting analogs were active (Table 20.4) (40). The Dmpa containing analog shows higher activity than any other analogs which contain Ppa, Mba, and Depa (Table 20.4). Since these four units have functional groups with different sizes and hydrophobicities, the bioactivity results can be used to understand optimal redundant properties for binding.

A methylated derivative of Ppa, β,β-(4-methylpentamethylene)-β-mercaptopropionic acid (4-MePpa) was also synthesized (Fig. 20.5). The two isomers (*cis* and *trans*) of this unit were incorporated into the place of Ppa in c[Ppa-D-Tyr(Et)-Phe-Val-Asn-Cys]-Pro-Arg-NH_2 (SK&F 101926). The resulting analogs are potent vasopressin V_1 receptor antagonists which also show some agonist activity (45). The resulting trans-isomer analog exhibited more agonist activities than the cis-isomer. However, the agonist activities of cis-isomer containing analogs were considerably less than the corresponding unsubstituted analogs. These results indicated that small changes in the structure of constrained units could provide desirable pharmacological properties.

Table 20.4 Binding Assays of Vasopressin Analogs

Analog	K_d (nM)*
c[Ppa-Phe(NO_2)-Phe-Val-Asn-Cys]-Pro-Lys-D-Tyr-NH_2	3.2 ± 0.7
c[Mab-Phe(NO_2)-Phe-Val-Asn-Cys]-Pro-Lys-D-Tyr-NH_2	27 ± 4.6
c[Dmpa-Phe(NO_2)-Phe-Val-Asn-Cys]-Pro-Lys-D-Tyr-NH_2	1.8 ± 0.6
c[Depa-Phe(NO_2)-Phe-Val-Asn-Cys]-Pro-Lys-D-Tyr-NH_2	2.55 ± 0.12

*The K_d denotes the dissociation constant calculated from the binding assays of vasopressin analogs to the V_1 receptor.

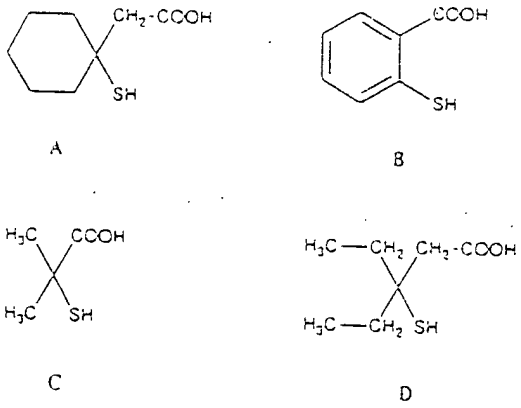


Fig. 20.4 Desaminocysteine derivatives. A, β,β-pentamethylene-β-mercaptopropionic acid (Ppa); B, 2-mercaptobenzoic acid; C, β,β-dimethyl-β-mercaptopropionic acid (Dmpa) and β,β-diethyl-β-mercaptopropionic acid (Depa).

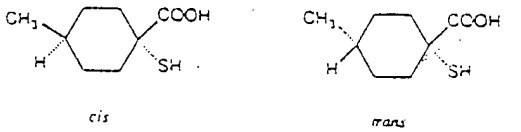


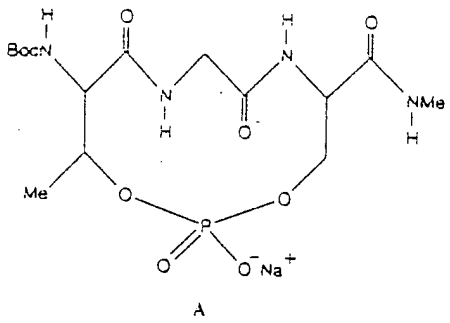
Fig. 20.5 Structures of *cis*- and *trans*-β,β-(4-methylpentamethylene)-β-mercaptopropionic acid (*cis*- and *trans*-4-MePpa).

2.3 Various Cyclic Linkages

The lactam and disulfide bridge formations have been the most popular linkages for cyclic structures. Several linkages have also been devised to connect various types of sidechain functional groups to peptide termini or other side chains. For example, to form a cyclic structure using two side-chain hydroxyl groups, a phosphodiester linkage (Fig. 20.6A) was proposed (46). In fact, this linkage occurs naturally in a protein, flavodoxin, isolated from *azotobacter*. Since the P(O) moieties can act as hydrogen-bonding or metal-binding acceptors, this linkage affects the conformational features of the resulting analogs. A disiloxane bridge has also been employed for linking two hydroxyl groups (47) and has been introduced into an enkephalin analog. The resulting cyclic enkephalin (Fig. 20.6B) has shown opioid activity.

A urethane linkage can be useful for connecting an amine to a side-chain hydroxyl group. Furthermore, the C(O)–NH bond of urethane has a high tendency to assume a cis configuration which can facilitate the formation of cyclic peptides. Wu and Kohn have synthesized Tyr-urethane containing pseudopeptides (Fig. 20.7) (48). An ester linkage connecting a carboxyl group and a hydroxyl group has been observed in many naturally occurring antibiotics. This linkage was used for the synthesis of a series of human renin inhibitors (49).

Cyclic linkages with aromatic rings also occur in nature. For example, an aryl ether linkage has been found in glycopeptide antibiotics (vancomycin) (50) and in the



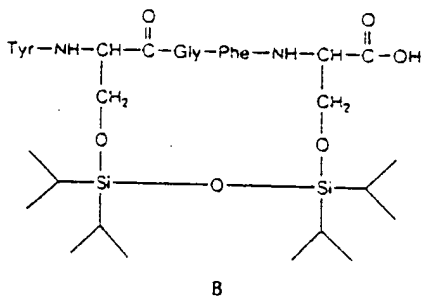


Fig. 20.6 Structures of A, a model peptide containing phosphodiester linkage; and B, disiloxane containing cyclic enkephalin.

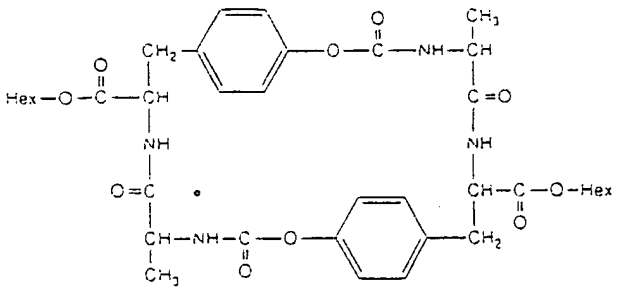


Fig. 20.7 Structure of urethane linkage-containing peptide.

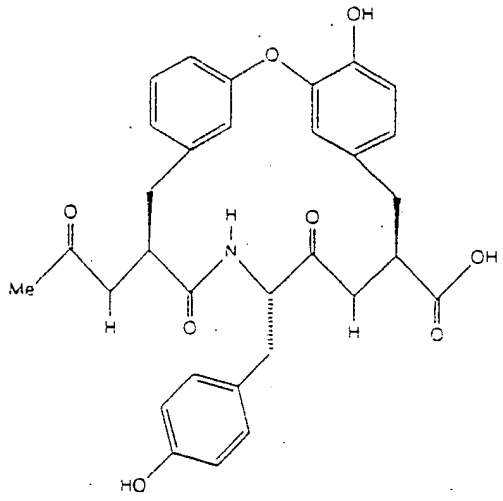


Fig. 20.8 Naturally occurring angiotensin converting enzyme inhibitor K-13.

naturally occurring angiotensin-converting enzyme inhibitor K-13 (Fig. 20.8) (51). Recently, Thaisrivongs et al. reported the connection of a histidine side chain to the *N*-terminus of a renin inhibitor by using the $-CH_2-C(O)-$ unit (Fig. 20.9) (52). The resulting analogs exhibited high binding affinity.

Recently, the lanthionine (monosulfide) bridge (53) which is observed in nisin and other peptide antibiotics, has been incorporated in the place of disulfide bridges in several bioactive peptides (54). The resulting analogs are more constrained than their disulfide-bridged counterparts because of their decreased ring size. Additionally, this modification increases the metabolic stability of the resulting analogs. When this modification was incorporated into cyclic enkephalins [i.e., Tyr-c(D-Ala_L-Gly-Phe-Ala_L)-NH_2 (55): Fig. 20.10], the resulting analogs showed bioactivities similar to those of their disulfide bridged counterparts [i.e., Tyr-c(D-Cys-Gly-Phe-Cys)-NH_2]. However, the half-life of the lanthionine enkephalin in the rat brain homogenates is much longer than that of the parent disul-

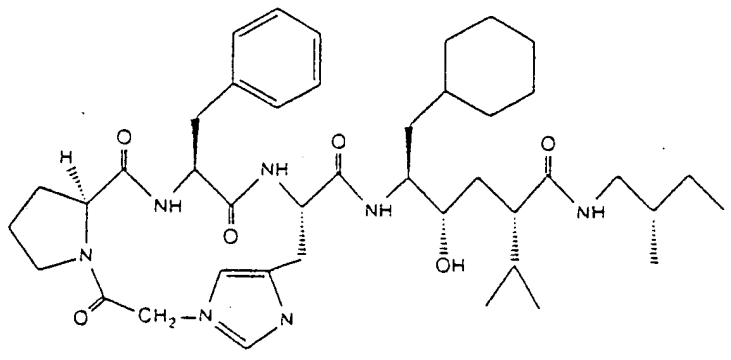


Fig. 20.9 A renin inhibitor in which the side chain of His is linked to N-terminus by $-CH_2-C(O)-$ unit.

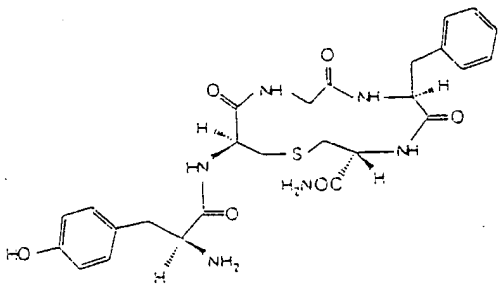


Fig. 20.10 Chemical structure of Tyr-c(D-Ala$_L$-Gly-Phe-Ala$_L$)-NH$_2$. This analog contains a lanthionine (monosulfide) bridge.

fide compounds. This structure is presently being incorporated into disulfide bridge containing peptides that include oxytocin and calcitonin as well as opioids.

2.4 Backbone Cyclizations and Bicyclic Structures

Gilon et al. have achieved the cyclization of peptides by linking an N" atom to either another N" atom, other side chains, the amino terminus or the carboxyl terminus using an appropriate linker (56). The possible cyclic structures are depicted in Figure 20.11. They also introduced this structure into a Substance P analog, Ac-Arg-Phe-Phe-Phe-Pro-Leu-Met-NH$_2$, leading to the structures shown in Figure 20.12. The analogs have similar biological profiles and show protracted activity in various tissues when compared to the parent analog (56). In the NMR studies of these two analogs, isomers containing a cis structure about the substituted amide bond were observed (57).

Bicyclic structures have been studied to avoid the flexibility of large cyclic peptides (58). When a bicyclic structure was formed in the oxytocin sequence, the resulting peptides showed high antagonist activity (59, 60). Also the parent monocyclic precursor possessed very little agonist activity. Multicyclic structures have also been considered as a method to stabilize an amphiphilic α-helical structure (61).

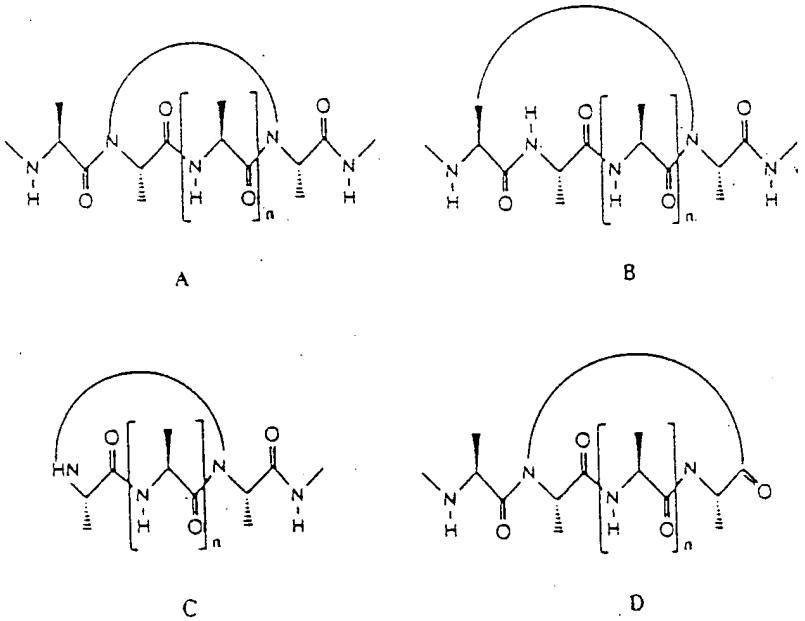
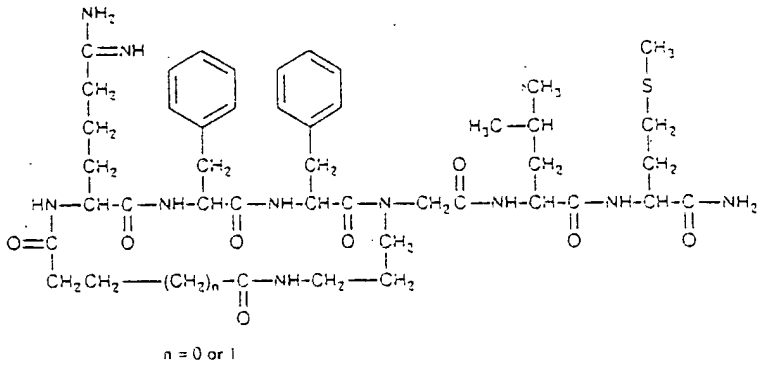


Fig. 20.11 Four possible backbone cyclizations. A, an N" atom to another N" atom; B, an N" atom to a side chain; C, an N" atom to amino terminus; or D, an N" atom to carboxylic terminus



n = 0 or 1

Fig. 20.12 Backbone cyclized substance P analogs. One analog has C'H$_2$ ($n = 1$) and another analog does not have this methylene ($n = 0$).

3 CONSTRAINED AMINO ACIDS

Constrained amino acids have been introduced into the sequences of bioactive peptides in order to obtain local constraints. Incorporation of these amino acids specifically restricts the rotation of N"–C", C"–C(O), C(O)–NH bonds, and side-chain conformations by covalent or noncovalent steric interactions. Thus, these amino acids can be used as conformational probes in an effort to understand local conformations responsible for the bioactivity of a particular peptide.

3.1 α-Methylated Amino Acids

Amino acids which are α-methylated have a structure in which the α-hydrogen atom is replaced by a methyl group. Methylation severely restricts rotation around the N–C" (ϕ) and C"–C(O) (ψ) bonds of amino acids. Figure 20.13 displays the changes in the allowed (ϕ) and (ψ) regions upon the α-methylation of glycine (Ala) (62). About 70% of the conformational space available to Gly cannot be adopted by Ala. Furthermore, about 90% of this conformational space is not available to α-aminoisobutyric acid [Aib or α-methylalanine (MeA); Fig. 20.14A (63–64).]

Among the α-methylated amino acids, Aib (observed in native channel peptides) is the most extensively studied. The conformational behaviors of Aib containing peptides were summarized in reviews by Venkataram Prasad, Balaram, and Karle (65, 66). Further studies have also been published (67–85). The conformational space preferentially adopted by Aib residues includes both the regions of left- and right-handed α and 3_{10} helices (86). This amino acid rarely exhibits extended structures (87). The Aib residue has been incorporated into numerous bioactive peptides including enkephalin (15, 88–91), angiotensin (92), bradykinin (93), chemoattractants (94), and substance P (95) in order to obtain highly active and selective analogs and to understand the conformations responsible for the recognition of their receptors.

Unlike Aib, all other α-methylated amino acids are chiral. Toniolo et al. reviewed the conformational preferences of isolvaline [Iva or L-α-ethyl-alanine (EtA); Fig. 20.14B], α-methylvaline [(αMe)Val], α-methylleucine [(αMe)Leu], and α-methylphenylalanine [(αMe)Phe; Fig 20.14C)] in peptides (96). The conformational preferences were determined by X-ray crystallographic analyses, ^{1}H-NMR spectroscopic studies, and conformational

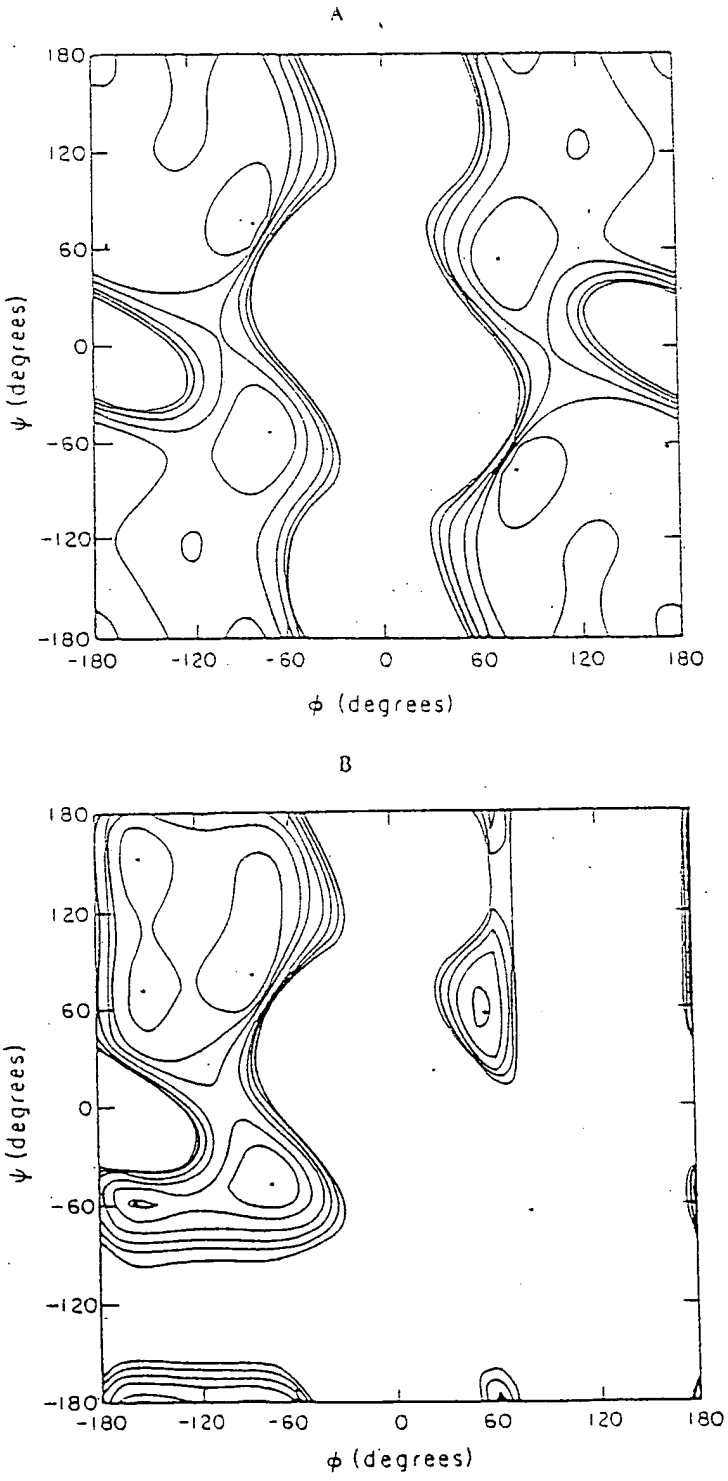


Fig. 20.13 Conformational energy contour map of (A), *N*-acetyl-*N'*-methyl-glycineamide; (B) *N*-acetyl-*N'*-methyl-alanineamide; and (C) *N*-acetyl-*N'*-methyl-α-amino-isobutyric acid amide.

C

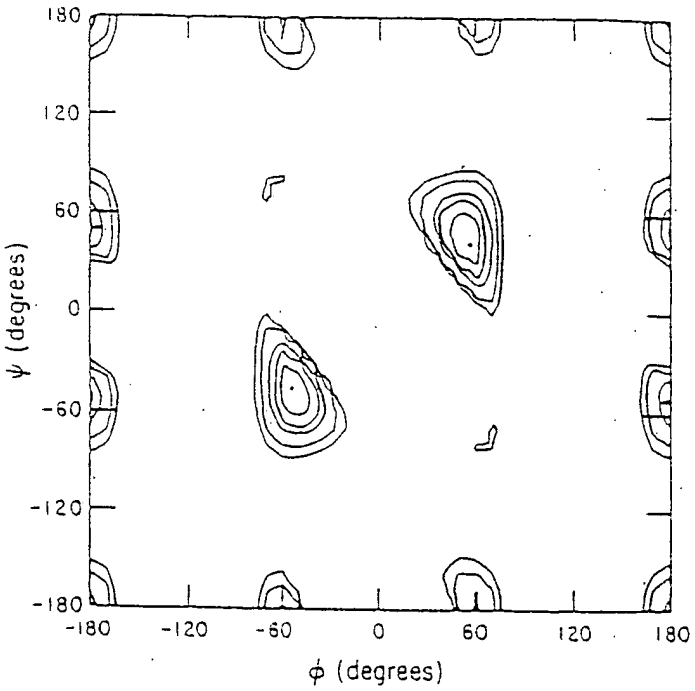
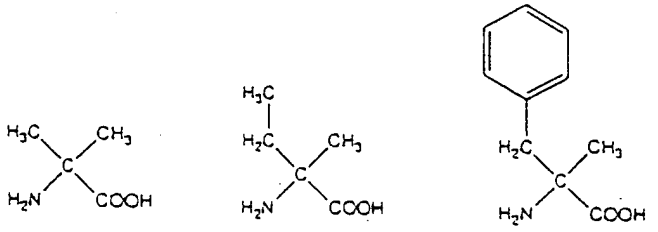


Fig. 20.13 (*Continued*)

energy calculations. In general, tripeptides and longer peptides containing these amino acids are folded in β-turns or in a 3_{10}-helical array. Furthermore, these amino acids rarely adopt the fully extended structure ($\phi = 180°$, $\psi = 180°$). Recently, the crystal state conformational analysis of homopeptides from [D-(αMe)Phe]$_n$ ($n = 1$, 2, 3, and 4) was reported (97). All of the (αMe)Phe residues were found to prefer torsion angles in the helical region of ϕ, ψ conformational map.

These amino acids have also been incorporated into bioactive peptides. For example, the (αMe)Phe containing cyclic dermorphin analog, Tyr-c[D-Orn-(αMe)-Phe-Glu]-NH_2, shows activities similar to the Phe containing parent analog (98). On



A B C

Fig. 20.14 Structures of α-methylated amino acids. A, α-aminoisobutyric acid (Aib); B, isovaline (Iva) (α-ethylalanine); and C, α-methylphenylalanine (αMePhe).

the contrary, the incorporation of (αMe)Tyr into enkephalin leads to an inactive analog (99). When (αMe)Phe is incorporated into a chemoattractant, the resulting compound shows reduced activity (100).

In addition, other α-methylated amino acids were studied resulting in the syntheses of (αMe)Arg (101), (αMe)Pro (102), (αMe)Orn (102), and (αMe)Ser (103). The X-ray studies of a (αMe)Ser-containing peptide indicated that this residue favorably adopts a conformation in which $\phi = -55°$ and $\psi = -32°$ (104).

3.2 α,α′-Dialkylglycines and α-Aminocycloalkane Carboxylic Acids

The replacement of the two hydrogens on the C^{α} atom of a glycine residue with two identical alkyl or aryl groups results in α,α-disubstituted glycines (Fig. 20.15) (105, 106) with the Aib residue having the simplest structure among this type of amino acids. As mentioned in the previous section, allowable ϕ and ψ angles for this residue are restricted to the α- or 3_{10}-helix regions. However, diethylglycine (Deg) and dipropylglycine (Dpg) residues exhibit different conformational preferences (Fig. 20.15A). These residues preferentially adopt fully extended structures where ϕ and ψ angles are both 180° (107, 108). A similar extended structure was also observed as a minimum energy conformation from the conformational studies of diphenylglycine (Dϕg; Fig. 20.15B (109). The dibenzylglycine (Dbg; Fig. 20.15C) residue of the tripeptide Gly-Dbg-Gly readily adopts conformations where the ϕ and ψ angles are (180°, 90°) and (180°, 270°) (110).

The α-aminocycloalkanecarboxylic acids ($Ac^{n}c$, $n = 3$–7) are also α,α-dialkylglycines with cyclized side chains incorporated into a cyclic structure (Fig. 20.16A). For example, α-aminocyclopropane carboxylic acid ($Ac^{3}c$) (111) contains a three-membered ring including the α-carbon. Several peptides containing $Ac^{3}c$ were examined by X-ray crystallography. In the solid state, this residue assumes a β-turn conformation or a distorted 3_{10} helix (112, 113). The conformational preference of other aminocycloalkane carboxylic acids such as α-aminocyclopentane carboxylic acid ($Ac^{5}c$) (114, 115), α-aminocyclohexane carboxylic acid ($Ac^{6}c$) (116), and α-aminocycloheptane carboxylic acid ($Ac^{7}c$) (117) are also similar.

It is interesting that although dialkylglycines and aminocycloalkane carboxylic acids have the same number of side-chain carbons, they show different conformational preferences. For example, $Ac^{5}c$ and $Ac^{7}c$ prefer a folded conformation, whereas Deg and Dpg favor a fully extended structure. This comparison demonstrates the substantial effects of cyclic side chains

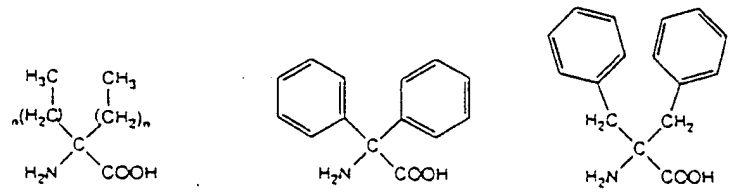


Fig. 20.15 Structures of α,α-dialkylated glycines. A, α-aminoisobutyric acid ($n = 0$, Aib); α,α-diethylglycine ($n = 1$, Deg) and α,α-dipropylglycine ($n = 2$, Dpg); B, α,α-diphenylglycine (Dϕg); and C, α,α-dibenzylglycine (Dbg).

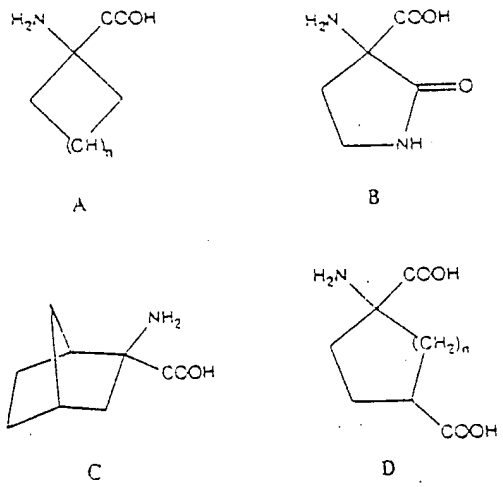


Fig. 20.16 Structures of A, aminocycloalkane carboxylic acids; α-aminocyclopropane carboxylic acid (Ac^3c, $n = 0$), α-aminocyclobutane carboxylic acid (Ac^4c, $n = 1$), α-aminocyclopentane carboxylic acid (Ac^5c, $n = 2$), α-aminocyclohexane carboxylic acid (Ac^6c, $n = 3$) and α-aminocycloheptane carboxylic acid (Ac^7c, $n = 4$); and their modifications B, α-aminopyrrolidone carboxylic acid (Apc); C, α-aminonorborane carboxylic acid; and D, 1-aminocyclopentane-1,3-dioic acid ($n = 1$) and 1-aminocyclohexane-1,4-dioic acid ($n = 2$).

on the conformation of the amino acid in peptides.

The α-aminocycloalkanecarboxylic acids were incorporated into bioactive peptides including opioid peptides, chemotactic peptides, and sweeteners. When Ac^5c was incorporated into various positions of enkephalins, the resulting peptides showed higher *in vivo* activity than the parent enkephalin amide (15). The chemotactic analogs (HCO-Met-X-Phe-OMe) containing Ac^5c, Ac^6c, and Ac^7c showed higher activity than their parent peptides (94). The Aib containing analog is slightly less active and the Ac^3c containing analog is inactive. Aspartame analogs in which Ac^nc ($n = 3$–8) residues are incorporated (Asp-Ac^n-OMe) have been synthesized. The analogs with Ac^nc ($n = 3$–5) retained a sweet taste (118). On the contrary, Ac^nc and Ac^7c containing analogs are bitter and the Ac^8c containing analog is tasteless.

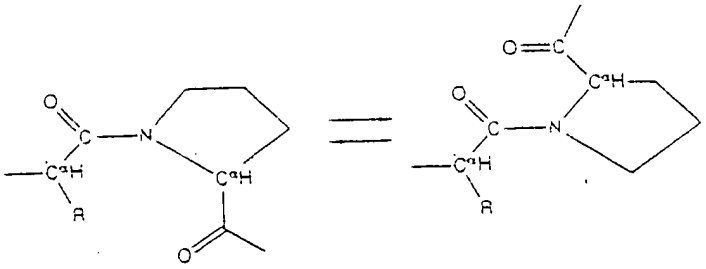
Recently, aminopyrrolidone carboxylic acid has been synthesized (Apc: Fig. 20.16B) (119). The structure of Apc is similar to Ac^5c except that one ethylene ($C^\beta H_2$-$C^\alpha H_2$) of the five-membered ring is replaced with an amide bond. Thus, the Apc residue contains a stereogenic center and the side-chain can be involved in hydrogen-bonding. When Apc was incorporated into valmuceptin, one of the resulting analogs, Tyr-(D,L)-Apc-Phe-Val-NH_2, showed opioid activity. On the other hand, the Ac^5c containing analog was inactive (120).

The α-aminonorborane carboxylic acids (Fig. 20.16C) can be classified as aminocycloalkane carboxylic acids (121, 122). Since these residues are bulky and highly constrained, they may be expected to show highly preferred conformations. In addition, analogs of glutamic acid were synthesized by placing the carboxyl group at the γ-position of Ac^5c and Ac^6c (Fig. 20.16D) (123).

3.3 N^α-C^α Cyclized Amino Acids

The N^α–C^α cyclized amino acids have been devised as modifications of proline. One of the most important characteristics of these amino acids is the occurrence of cis/trans isomerism (Fig. 20.17) (124). The tertiary amide bond of N^α–C^α cyclized amino acids leads to the cis and trans amide bonds with approximately 2 kcal/mole energy difference (125). This value is much lower than that of a normal peptide bond (approx. 10 kcal/mol). This type of cis/trans isomerization can be detected by NMR. The NMR studies of morphiceptin (Tyr-Pro-Phe-Pro-NH_2) have shown four different isomers generated from cis/trans isomerization of the Tyr-Pro and Phe-Pro amide bonds (126).

Since the N^α–C^α bond of prolinelike amino acids is included in the pyrrolidine ring, the ϕ angle of these amino acids is

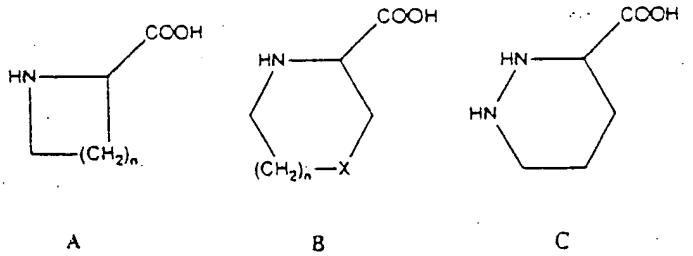


cis *trans*

Fig. 20.17 A cis/trans isomerization of proline.

highly restricted. The rotation of the C^{α}–C(O) bond is also constrained by the steric interaction between the pyrrolidine ring and C(O). These amino acids can affect the conformation of the preceding residue. The ψ angle rotation for the preceding L-residue is restricted to between 60 and 180°. When the Pro residue assumes a trans configuration, the steric interaction occurs between the side chain of the preceding L-residue and the $C^{\delta}H_2$ attached to the nitrogen of the Pro residue. In a cis isomer of proline, a steric overlap occurs between the side chain of the preceding L-residue and the C^{α} group of the Pro residue (127).

3.3.1 PROLINE MIMETICS WITH DIFFERENT RING SIZES. Structures such as Azy (azyline or aziridine-2-carboxylic acid), Aze (azetidine-2-carboxylic acid), and Pip (pipecolic acid) are proline analogs or mimetics with different ring sizes (Fig. 20.18A). The Azy and Aze have 3- and 4-membered rings, respectively; whereas the Pip contains a 6-membered ring. When one of the methylene groups in the cyclic structure is replaced by an oxygen or a sulfur, (O)Pro (oxazolidine-4-carboxylic acid), (O)Pip (perhydro-1,4-oxazine-3-carboxylic acid or 3-morpholinecarboxylic acid), (S)Pro (or Thz; thiazolidine-4-carboxylic acid), and (S)Pip (perhydro-1,4-thioxazine-3-carboxylic acid) are obtained (Fig. 20.18B). The conformational behavior of these amino acids is well-summarized in a review written by Toniolo (128). Recently, piperazic acid (Piz; Fig. 20.18C) was found as a component of the naturally occurring oxytocin antagonist c[Pro-D-Phe-(NOH)Ile-D-Piz-Piz-D-(NMe)Phe] (129).



A B C

Fig. 20.18 Proline mimetics with different ring size. A, aziridine-2-carboxylic acid (Azy, $n = 0$), azetidine-2-carboxylic acid (Aze, $n = 1$), proline (Pro, $n = 2$) and pipecolic acid (Pip, $n = 3$) and their modified forms; B, oxazolidine-4-carboxylic acid [$n = 0$, X = O; (O)Pro or Oxz], thiozolidine-4-carboxylic acid [$n = 0$, X = S; (S)Pro or Thz], perhydro-1,4-oxazine-3-carboxylic acid or 3-morpholine carboxylic acid [$n = 1$, X = O; (O)Pip and perhydro-1,4-thioxazine-3-carboxylic acid [$n = 1$, X = S; (S)Pip]; and C, piperazic acid (Piz).

The Aze residue was incorporated into collagen, the fibrous protein whose structure is characterized by a large population of proline and hydroxyproline. From theoretical calculations peptides containing Aze are somewhat more flexible than the corresponding peptides containing Pro (130–132). This occurrence can be explained by a decrease in repulsive noncovalent interactions between the rings of neighboring residues. Since the Aze residue is smaller than the Pro residue, the steric interaction between Aze residues is weaker than those between Pro residues. Thus, the replacement of Pro with Aze destabilizes the collagenlike triple helix (130–132).

Veber et al. incorporated Aze, Thz, and Pip in place of Pro in a somatostatin analog c(Pro-Phe-D-Trp-Lys-Thr-Phe) (133). The resulting analogs exhibited higher activity than the parent analog. The incorporation of (O)Pro, (S)Pro, and Pip in place of Pro in morphiceptin provided analogs with reduced activities (134). When D-Pip was incorporated at the same position, the resulting Tyr-D-Pip-Phe-Pro-NH_2 showed opioid activity (135), unlike Tyr-D-Pro-Phe-Pro-NH_2 (136). This result suggests that the Pip residue can adopt conformations not allowed for the Pro residue.

3.3.2 HIGHLY CONSTRAINED PROLINE MIMETICS. Highly constrained Pro analogs have been devised incorporating additional functional groups (Fig. 20.19). Methanoprolines, containing [5,3,0] and [5,4,1] bicyclic systems, have been prepared, including 2,4-methanoproline (2,4-MePro; Fig. 20.19A (137–139) 2,3-methanoproline (2,3-MePro; Fig. 20.19B (140) and 3,4-methanoproline (3,4-MePro; Fig. 20.19C (141). Methylation on a carbon of the proline ring produced 2-methylproline [(α-Me)Pro; Fig. 20.19D] (142) and 3-methylproline [(β-Me)Pro; Fig. 20.19E] (142). The ethylene group was incorporated in the structures of 4-aza-

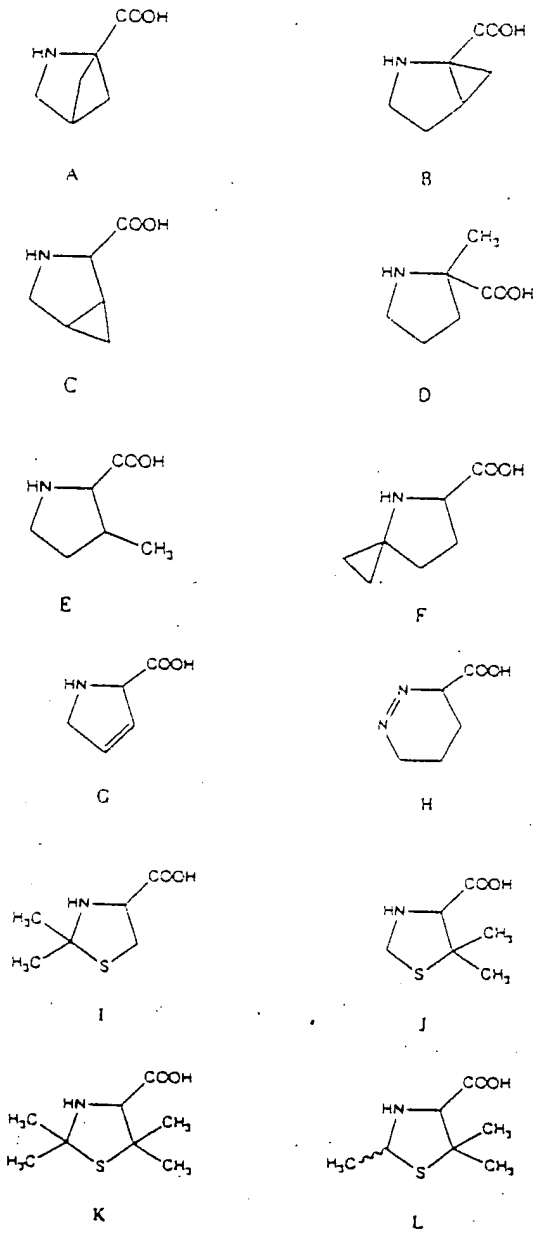


Fig. 20.19 Constrained proline mimetics. A, 2,4-methanoproline (2,4-MePro); B, 2,3-methanoproline (2,3-MePro); C, 3,4-methanoproline (3,4-MePro); D, 2-methylproline [(α-Me)Pro]; E, 3-methylproline [(β-Me)Pro]; F, 4-azaspiro[2,4]heptane carboxylic acid; G, 3,4-dehydroproline (3,4-ΔPro); H, dehydropiperazic acid (ΔPiz); I, 2,2-dimethylthiazolidine-4-carboxylic acid (2,2-Dtc); J, 5,5-dimethylthiazolidine-4-carboxylic acid (5,5-Dtc); K, 2,2,5,5-tetramethylthiazolidine-4-carboxylic acid; and L, 2,5,5-trimethylthiazolidine-4-carboxylic acid.

spiro[2,4]heptane carboxylic acid (Fig. 20.19F) (141). In 3,4-dehydroproline (3,4-ΔPro; Fig. 20.19b (134) and dehydropiperazic acid (ΔPiz; Fig. 20.19H) (129, 143–145), one of the bonds in the ring was replaced with a double bond. In addition, methyl groups were introduced into the thiazolidine structure and the resulting 2,2- or 5,5 -dimethylthiazolidine -4-carboxylic acid (Dtc; Fig. 20.19I, J) have been extensively studied (146). Recently, 2,2,5,5 - tetramethylthiazolidine - 4 - carboxylic acid was synthesized (Fig. 20.19K) and two isomers of 2,5,5-trimethylthiazolidine-4-carboxylic acid (Fig. 20.19L) (120) were synthesized.

Scheraga et al. carried out conformational studies of 2,4-MePro containing peptides using NMR, X-ray crystallographic, and computational techniques (138, 139). The NMR studies of Ac-2,4-MePro-NHMe and Ac-Tyr-2,4-MePro-NHMe in aqueous solution indicate that the Ac-2,4-MePro and Tyr-2,4-MePro amide bonds adopt a trans structure to an overwhelming extent. This result suggests that the bicyclic structure generated by the incorporation of a methylene group into a Pro residue stabilizes the trans amide bond. The energy difference between cis- and trans-forms was calculated to be 5.9–8.8 kcal/mol. The additional methylene group also perturbs backbone conformational angles. The calculation of the interior torsion angle ϕ of this amino acid showed two minima at $\pm 29°$, rather than one at $-70°$, as seen with the Pro residue. Similarly, optimal values for the ψ torsional angle are also perturbed. The average ψ values of this amino acid are $-50°$ and $90°$, whereas those of the Pro residue are $-19°$, $75°$, and $160°$. In the crystalline state, the observed (ϕ, ψ) angles for Ac-2,4-MePro-NHMe and its enantiomer are $(-29°, 114°)$ and $(29°, -114°)$, respectively.

Marshall and his associates incorporated this amino acid in place of each of three Pro residues in bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), one at a time. In spite of the desirable effects of 2,4-MePro which stabilize the trans configuration of the Pro-Pro and Ser-Pro amide bonds, the resulting analogs showed significantly reduced activities (137). An explanation for the reduced activities can be suggested by postulating that the additional methylene of the 2,4-MePro is involved in conformational changes of the residue and the steric interactions with the receptor. Marshall's group also incorporated this amino acid into angiotensin II (Asp-Val-Try -Val-His-Pro-Phe) providing an analog with 26% of potency of the parent molecule.

Stammer et al. synthesized 2,3-MePro and carried out conformational studies (140). In the X-ray structure of Ac-(2R,3S)-MePro-NHMe, the ϕ and ψ torsion angles are 76° and 7°, respectively, and the Ac-(2R,3S)-MePro bond is in a cis conformation. The ϕ and ψ angles were found to be essentially the same as those of Ac-D-Pro-NHMe. The NMR studies of this model compound indicate the existence of the two stereoisomers. A somewhat greater preference for the cis form of the Ac-2,3-MePro amide bond was observed. This result was supported by the above X-ray structure and the relative energies of two isomers. The cis isomer of Ac-2,3-MePro-NHMe was favored over the trans isomer by 1.4 kcal/mol from the calculations. When a taste ligand was synthesized using this amino acid, the resulting compound [Asp-(2*S*,3*R*) -2,3-MePro-OPr] was bitter (147). The NMR studies of this compound suggest that the Asp-(2*S*,3*R*)-MePro amide bond adopts only a cis configuration. The comparison of the relative energies of the fully minimized cis and trans isomers of Asp-(2*S*,3*R*)-MePro-OPr demonstrate a 2.1 kcal/mol energy difference favoring the cis conformation.

Delaney and Madison carried out conformational studies of Ac-(αMe)Pro-NHMe and Ac-(βMe)Pro-NHMe (142). In the NMR studies, the Ac-(αMe)Pro-NHMe

did not exhibit a cis isomer in any solvent, while both *anti*- and *syn*-(βMe)Pro-NHMe (*anti*; the methyl group is on the opposite side of the proline ring from the carboxamide, *syn*; on the same side) exhibited 15% to 25% of the cis isomer in different solvents. The absence of a cis isomer of Ac-(αMe)Pro-NHMe may be caused by the steric interactions between the α-methyl and acetyl–methyl groups.

The conformational behaviors of Ac-*anti*-(βMe)Pro-NHMe are nearly identical with those of Ac-Pro-NHMe in the same solvents (142). Specifically, the C_7 conformer (γ-turn; $\phi = -80°$, $\psi = 80°$) dominates the conformational populations in nonpolar solvents. A mixture of C_7, right-handed α helix, and polyproline II ($\phi = -80°$, $\psi = 150°$) conformers was observed in acetonitrile. On the other hand, in water, the polyproline II conformation predominated. For Ac-*syn*-(βMe)Pro-NHMe, the γ-turn can be destabilized by steric interactions between the methyl group and the carbonyl oxygen of the Pro residue.

In addition, the hydrogen-bonding in the C_7 conformer of Ac-(αMe)Pro-NHMe (between C(O) of the acetyl group and NH of the terminal methylamide) is unusually strong as shown by IR spectroscopy (142). A significant amount of this intramolecular hydrogen-bonding is retained in aqueous solution. These results indicate that the α-methyl group stabilizes the γ-turn of this model peptide by inducing a fold of the mainchain to avoid methyl eclipsing interactions. The α-methylated structure also shields the peptide hydrogen bond from solvent. The stabilization of the γ-turn by the α-methylation of Pro was also observed in the conformational studies of (αMe)Pro containing bradykinin (148) and other bioactive peptides (149). The (αMe)Pro was incorporated into a renin inhibitor producing a highly active analog, Boc-(αMe)Pro-Phe-His-Leuψ[CHOHCH$_2$]Val-Ile-Amp (150).

Samanen et al. examined the conformational preferences of 5,5-dimethylthiazoline-4-carboxylic acid (Dtc) (146). The ^{1}H-NMR spectrum of Boc-Dtc-Ile-OMe displayed two stereoisomers resulting from the cis/trans isomerization of the Boc-Dtc bond. On the other hand, the X-ray crystal structure of Boc-Dtc-OH showed only a cis configuration of the urethane amide bond. Conformational studies using Ac-Dtc-NHMe suggest that the steric interaction between the *syn*-β-methyl group and the carbonyl group of Dtc destabilized the hydrogen-bonding between the Boc C(O) and the NH of the terminal amide of the C_7 conformation. This γ-turn conformation is the predominant conformation for Ac-Pro-NHMe. Instead, the Dtc residue adopts conformations in which ψ is 110–150° or 320–360°. These results suggest that the substitution of Dtc for Pro may test the functional importance of the C_7 conformation in a position of a bioactive peptide. When this amino acid was incorporated into position 5 of an angiotensin II (Sar-Arg-Val-Tyr-X-His-Pro-Ile-OH) analog (92, 151) the resulting analog showed higher activity than the Pro containing analog. This residue was also introduced into cholecystokinin. An R-Dtc containing analog showed high bioactivity (152).

Other constrained Pro analogs have been incorporated into bioactive peptides. For example, 3,4-dehydroproline (ΔPro) was introduced into somatostatin (133), morphiceptin (134), and oxytocin (153) in place of Pro. The resulting analogs displayed higher activity than the parent compounds. When ΔPiz was incorporated in place of Piz of the naturally occurring oxytocin antagonist, the resulting analog was highly active (115–117, 121).

The introduction of additional groups into the proline structure also provides substantial preferences for their conformations and cis/trans structures at the preceding amide bond. Thus, these proline mimetics can be useful in the design of biologically active peptide analogs since they affect local conformations and cis/trans preferences of the amide bonds.

3.3.3 PYROGLUTAMIC ACIDS AND HYDROXY PROLINE ANALOGS. Pyroglutamic acid (pGlu) also contains a N^{α}–C^{α} cyclized structure, but it is formed by *N*-acylation (Fig. 20.20A). This novel amino acid derivative is present in many naturally occurring peptides and has been incorporated into bioactive peptide analogs (154–158). Molecular mechanical calculations for L-pGlu-NHMe indicate that this residue adopts a folded conformation (159). However, the X-ray structure of L-pGlu-NHMe has displayed an extended conformation. A highly constrained derivative of this residue, 2,3-MepGlu: Fig. 20.20B) was also prepared to increase the metabolic stability of thyrotrophin releasing hormone (TRH). The resulting 2,3-MepGlu containing TRH analog is significantly more stable to enzymatic degradation than TRH. The X-ray crystal structure of 2,3-MepGlu-NHMe displayed that the (ϕ, ψ) angles of this residue are (−143°, −16°) (159).

Naturally occurring hydroxyproline has also been modified. These synthetic studies include the introduction of an additional hydroxyl group (i.e., 3,4-dihydroxy proline; Fig. 20.20C); changes in ring size (i.e., 3,4-dihydroxy pipercolic acid: Fig. 20.20D) (160) and alkylation of a hydroxy group (alkoxy proline: Fig. 20.20E) (161). These analogs represent interesting mimetics which can be incorporated into bioactive peptides.

3.4 N^{α}-Methylated Amino Acids

The N^{α}-methylated amino acids are commonly found in naturally occurring peptide antibiotics. Since the methylation of N^{α} eliminates the hydrogen on the N^{α} atom, the hydrogen-bonding pattern of peptides containing these amino acids are different from that of the unmethylated peptides. The *N*-methylated amide bond often adopts a cis as well as a trans geometry as with the N^{α}–C^{α} cyclized amino acid derivatives above. Conformational calculations and NMR studies on Sar-Sar (Sar: sarcocine or N-methylglycine) dipeptide showed that the two forms are nearly isoenthalpic; the cis isomer is higher in energy only by 0.6 kcal/mol (162). Amino acid *N*-methylation also affects the rotation of ϕ and ψ angles. The allowed conformational space of Ac-Ala-NHMe in a trans configuration is reduced when a methyl group is incorporated (Fig. 20.21A). The allowed conformational space for the cis isomer of Ac-(NMe)Ala-NMe is also depicted (Fig. 20.21B). The ϕ and ψ angles of the two

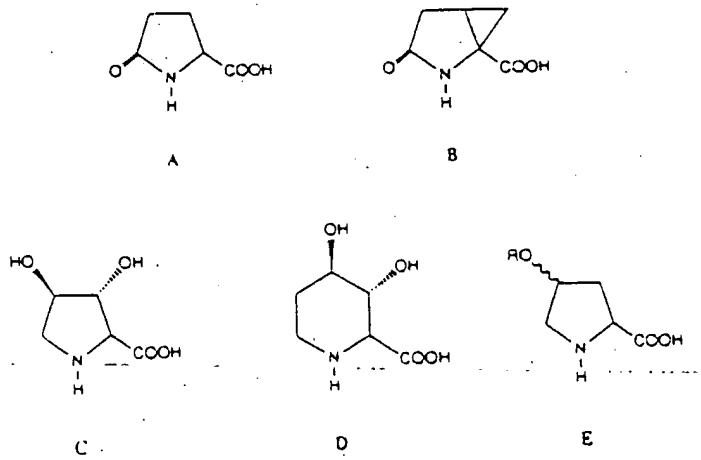


Fig. 20.20 Structures of A, pyroglutamic acid (pGlu); B, 2,3-methanopyroglutamic acid (2,3-MepGlu); C, 3,4-dihydroxyproline; D, 3,4-dihydroxypipercolic acid; and E, 4-alkoxyproline

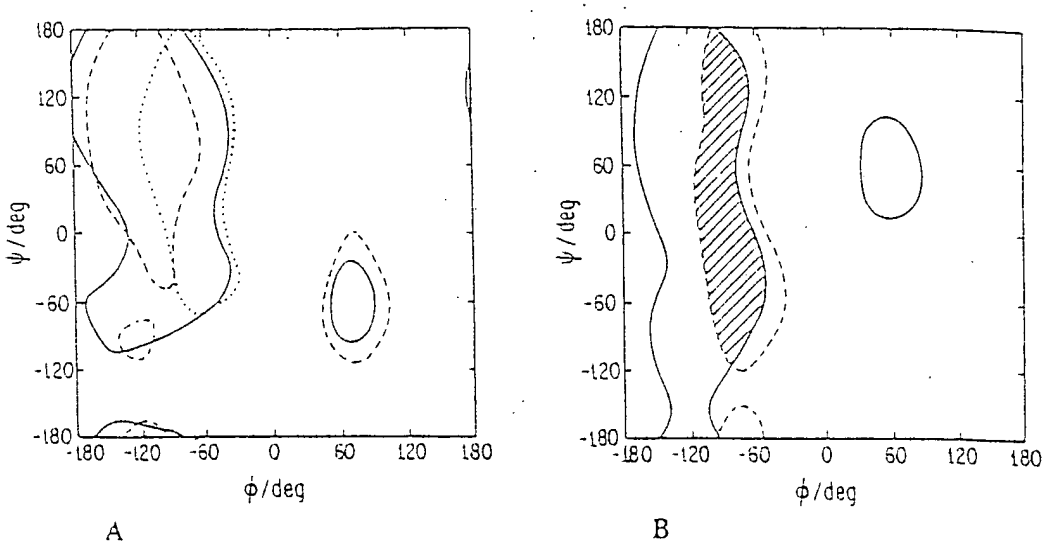


Fig. 20.21 Conformational energy contour map of A Ac-Ala-NHMe (solid), Ac-(NMe)Ala-NHMe (dashed) and Ac-Pro-NHMe (dotted) in a trans configuration of the amide bond; and B, Ac-(NMe)Ala-NHMe (solid) and Ac-Pro-NHMe (dashed) in a cis configuration of the amide bond.

lowest energy conformations for this isomer are approximately (−70°, 140°) and (−70°, −40°), respectively. These are similar to those for the Pro residue (163, 164).

The *N*-methylated amino acid can affect the rotation of the C"–C(O) bond of the preceding residue. The ψ angle of any L-amino acid residue which immediately precedes a N^{α}-methylamino acid is restricted to a range from 60° to 180°. When the *N*-methylated amino acid assumes a trans configuration, a range of $-180° < \psi < 0°$ is precluded by steric overlap involving the side chain of the preceding L-residue with the methyl group attached to the nitrogen. The same ψ angle range is also precluded for the L-residue followed by a cis conformer of the *N*-methylated amino acid because of steric overlaps between the side chain of the L-residue and the C"H group of the *N*-methyl amino acid. In addition, when the residue preceding the *N*-methyl amino acid is a β-substituted or branched amino acid, the torsion angle for the side chain conformation (χ) is also severely restricted by the N^{α}-methylated residues (163, 164).

The above conformational restrictions of *N*-methylated amino acids has been used to help understand the molecular basis of the bioactivities of morphiceptin and dermorphin (163, 165). The biologically important functional groups of these two peptide opioids involve the amino and phenolic groups of the Tyr^1 residue and the aromatic group of the Phe^3 residue. The relative spatial arrangements of these functional groups can be defined by a set of eight torsion angles: ψ^1, χ_1^1 and ω^1 of Tyr^1, ϕ^2, ψ^2, and ω^2 of Pro^2 or D-Ala^2, ϕ^3 and χ_1^3 of Phe^3. To estimate each of these angles for morphiceptin bioactivity, a series of analogs were designed in which *N*-methyl amino acids were systematically incorporated into morphiceptin and dermorphin tetrapeptides. The resulting analogs have showed a correlation between bioactivity and distinct conformational preferences. The biologically meaningful value of each torsion angle was specifically identified by comparing its accessible space and bioactivities of the corresponding analog. Similar methods were applied for dermorphin. From these studies, conforma-

tions that are responsible for the bioactivities of morphiceptin and dermorphin have been postulated (163, 165).

Aubry and Marraud studied the effects of *N*-methylation on the β-turn structure by examining crystal structures of ten *N*-methylated R-C(O)-X-(NMe)Y-NHR dipeptides (Table 20.5) (166). Five of these analogs contained homochiral X and Y residues, two contained a heterochiral sequence and three contained one or two Gly residues. The distances between the α-carbons of the X and Y residues of methylated peptides in the trans configuration were found to be similar to those of the non-*N*-methylated peptides. However, the distances of the cis isomer are about 1 Å shorter. Aubry and Marraud also found that the β-turn of heterochiral dipeptide sequences are not perturbed by *N*-methylation and retain the same extent of β-folding. On the other hand, homochiral sequences preferentially have adopted a different β-turn conformation upon *N*-methylation.

In addition to peptide opioids (163, 165, 167, 168), these amino acids were incorporated into other bioactive peptides such as bradykinin (169), an opioid antagonist (CTOP) (170), TRH (164), angiotensin II (92), luteinizing hormone-releasing hormone (LHRH) (171), and CCK_8 (172).

3.5 β- and γ-Amino Cycloalkane Carboxylic Acids

The Pro residue allows a cis/trans isomerization at the preceding amide bond. There is difficulty in analyzing conformations where two or more distinct but interchanging structures are made up of all-trans isomer and isomers with at least one cis amide bond coexist. To avoid the possibility of such cis/trans isomerization, β-aminocyclopentane carboxylic acid (β-Ac^5c: Fig. 20.22A) was incorporated into peptides (173–175). This amino acid is similar to Pro but the preceding amide bond can adopt only a trans configuration since it contains an exocyclic amine. The residue contains an extra stereogenic center. Thus, four different isomers (1*S*,2*R*, 1*S*,2*S*, 1*R*,2*R*, and 1*R*,2*S*) are available. When these isomers are incorporated, each isomer imparts conformational effects on the peptide structure. Thus, the comparisons between the results of conformational analyses and bioassays of the four analogs containing these isomers can provide useful

Table 20.5 Conformations of *N*-Methylated Amino Acid-Containing Dipeptides

Compound	ϕ_1[a]	ψ_1[a]	ω_1[a]	ϕ_2[a]	ψ_2[a]	Conformation
*t*Bu-C(O)-Pro-(NMe)Ala-OMe	−70	153	176	−92	157	
*t*Bu-C(O)-Pro-(NMe)Ala-NHiPr	−62	135	−13	119	60	βVI-turn
*t*Bu-C(O)-Pro-(NMe)Leu-NHMe	−66	146	−4	−108	55	βVI-turn
*t*Bu-C(O)-Pro-(NMe)Phe-NHMe	−63	146	4	−113	52	βVI-turn
*t*Bu-C(O)-Ala-(NMe)Ala-NHiPr	−66	137	−1	−113	48	βVI-turn
*t*Bu-C(O)-Pro-D-(NMe)Ala-NHMe	−58	136	−178	97	−19	βII-turn
*t*Bu-C(O)-Ala-D-(NMe)Ala-NHMe	−61	129	179	99	−23	βII-turn
*t*Bu-C(O)-Pro-(NMe)Gly-NHiPr	−56	135	−178	96	−17	βII-turn
*t*Bu-C(O)-Ala-(NMe)Gly-NHiPr	−71	153	171	103	−148	
*i*Pr-C(O)-Gly-(NMe)Gly-NHiPr	−73	163	174	−90	−176	

[a]The unit for all the torsion angles is degrees

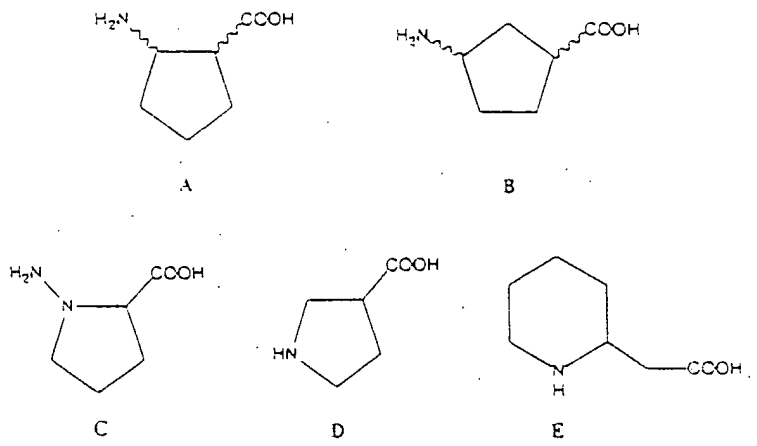


Fig. 20.22 Structures of A, β-aminocyclopentane carboxylic acid (β-Ac^{5}c); B, γ-aminocyclopentane carboxylic acid (γ-Ac^{5}c); C, aminoproline (aminoPro; D, β-proline (βPro); and E, piperidineacetic acid.

information about the conformations responsible for bioactivity.

When β-Ac^{5}c was incorporated into the second position of morphiceptin, only the (1*S*,2*R*) isomer containing analogs were active (173, 175). The conformational analysis of these analogs provided a preferred conformation which is similar to the structure suggested from the studies of other morphiceptin analogs (163, 165). Also these amino acids have been incorporated into somatostatin and taste ligands (174). Although the resulting somatostatin analogs were inactive, their conformational analyses provided useful information about the molecular basis for somatostatin bioactivity. The taste of Asp-β-Ac^{5}c-OMe is dependent on the configurations of the β-Ac^{5}c. For the trans-β-Ac^{5}c containing molecules, the 1*R*,2*R*-analog is sweet, where the 1*S*,2*S*-analog is bitter. For the analogs with cis-β-Ac^{5}c, the 1*S*,2*R*-analog is sweet, whereas the 1*R*,2*S*-analog is tasteless. The results of the conformational studies for these taste ligands were consistent with our model for the molecular basis of taste (174).

Also synthesized was γ-aminocyclopentane carboxylic acid (γ-Ac^{5}c; Fig. 20.22B) (173), 1-aminoproline (aminoPro; Fig. 20.22C), and β-proline (β-Pro; Fig. 20.22D) (120, 176). These proline analogs have been incorporated into morphiceptin. The γ-Ac^{5}c and aminoPro containing analogs are inactive., but the bioactivity of β-Pro-containing analogs are dependent on the configuration of that residue (120). Piperidineacetic acid (Fig. 20.22E) has also been synthesized and is also useful as a peptidomimetic residue in constrained peptides (177).

3.6 α,β-Unsaturated Amino Acids

In α,β-unsaturated amino acids (dehydro amino acids) (178), a double bond exists between C^{α} and C^{β} (Fig. 20.23). These residues have been found naturally in antibiotics of microbial origin and in some proteins. Singh et al. extensively studied a number of small peptides containing dehydrophenylalanine(s) (ΔPhe) and dehydroleucine (ΔLeu) using X-ray crystallography (179–184). In most cases, these dehydro residues induce β-turn structures in the peptide backbone by promoting hydrogen-bonding between C(O) of the residue *i* and NH of the residue *i* + 3 (Fig. 20.24) (185). The values of ϕ and ψ are close to 80 and

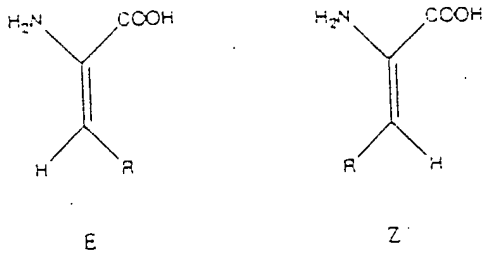


Fig. 20.23 Structures of dehydroamino acid in *E* and *Z* forms.

0°, respectively, when the ΔPhe residue is placed in the $(i + 2)$ position of the β-turn. If these residues occur at the $(i + 1)$ position, the ϕ and ψ are centered around −60 and 120°, respectively.

In the cases of Ac-ΔPhe-OH or Ac-ΔPhe-OEt, the ϕ values are close to either −60 or 80°. These values suggest a marked preference of ΔPhe for the two conformations in which ϕ and ψ are −60 and 120° or 80 and 0° (180). Similar results were obtained from the NMR and IR spectroscopic studies of Boc-X-ΔPhe-NHMe (X = Ala, Gly, Pro, Val) (186). From the X-ray-crystallographic studies of Ac-Pro-ΔVal-NHMe, a slightly different β-turn was observed. This peptide adopts the torsion angles, $\phi_1 = -68.3°$, $\psi_1 = -20.1°$, $\phi_2 = -73.5°$ and $\psi_2 = -14.1°$ (187). This conformation is characterized as a β-turn structure between

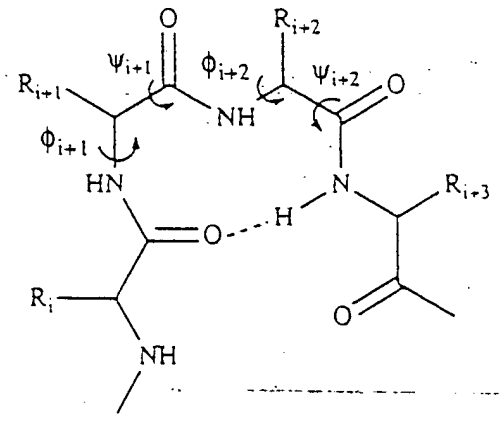


Fig. 20.24 Typical β-turn structure.

the type I and the type III conformations. The NMR and IR studies of model peptides, homochiral Ac-Pro-Y-NHMe (Y = Val, Phe, Leu, Abu), and heterochiral Ac-Pro-D-Y-NHMe, as well as α,β-unsaturated Ac-Pro-ΔY-NHMe [ΔY = ΔVal, (Z)-ΔPhe, (Z)-ΔLeu, (Z)-ΔAbu] were carried out (188). The homochiral compounds are conformationally flexible and display an inverse γ-turn, a β-turn and open forms in an equilibrium depending on the nature of the Y residue side chain. However, the heterochiral and α,β-dehydropeptides display type II β-turns as the dominating secondary structures (188).

The crystal structure of the linear tetrapeptide, Boc-Leu-ΔPhe-Ala-Leu-OCH_3, exhibits a double turn in which the type III and type I β-turns occur consecutively using ΔPhe as a common corner residue (189). This structure contains Leu ($\phi = -54.1°$, $\psi = -34.5°$) and ΔPhe ($\phi = -59.9$, $\psi = -17.1°$) as the corner residues of the type III β-turn, and ΔPhe ($\phi = -59.9°$, $\psi = -17.1°$) and Ala ($\phi = -80.4°$, $\psi = 0.5°$) as the corner residues for the type I β-turn. Furthermore, two intramolecular 4-1 type hydrogen bonds were observed to stabilize this turn structure. The above torsion angles indicate that the overall structure of this peptide is helical. Similar 3_{10} helical structures induced by consecutive β-turns are observed in other peptides containing ΔPhe, including Z-D-Ala-ΔPhe-Gly-NH_2 (190) and Ac-ΔPhe-Val-OH (191).

When peptides contained two ΔPhe residues, such helical structures were more clearly observed. For example, the peptide Ac-ΔPhe-Ala-ΔPhe-NHMe adopts a right-handed 3_{10}-helical conformation (192). The two consecutive 10-membered rings are formed by two hydrogen bonds between acetyl C(O) and NH of ΔPhe^3 and between C(O) of ΔPhe^1 and NH of the *N*-methyl amide. In the solid state, the conformation of Boc-D-Ala-ΔPhe-Gly-ΔPhe-D-Ala-OMe is characterized by the presence of two type III β-turns. Thus, this peptide assumes a

left-handed 3_{10}-helical conformation. The left-handed sense is due to the D-Ala residues (193). In the crystal structures of Boc-Gly-ΔPhe-Leu-ΔPhe-Ala-NHMe (194) and Boc-Ala-ΔPhe-ΔPhe-NHMe (195), 3_{10}-helical structures were observed. Recently, Chauhan et al. reported a crystal structure of Boc-Val-ΔPhe-Ala-Phe-Ala-Phe-ΔPhe-Val-ΔPhe-Gly-OMe (196) which showed seven consecutive type III β-turns formed by seven 4-1 intramolecular hydrogen bonds. Thus, the overall conformation is a right-handed 3_{10} helix with three complete helical turns.

The ΔAla residue has different conformational preferences from those observed in ΔPhe and ΔLeu. In the crystal structure of Boc-Phe-ΔAla-OMe, the ϕ and ψ angles of the ΔAla residue are −170 and 178° (197). The NMR studies of the other ΔAla containing peptide, Boc-X-ΔAla-NHMe (X = Ala, Val and Phe), indicate that the ΔAla induces an inverse γ-turn around the preceding residue of the ΔAla (X residue) (198). The ϕ and ψ angles of the preceding residue of ΔAla are −70 and 70°, respectively. The interproton distance between $C^{\alpha}H$ of the $i+1$ residue and NH of the $i+2$ residue is 2.5 Å. Unlike the structure of Boc-Val-ΔAla-NHMe in solution, the corresponding saturated analog, Boc-Val-Ala-NHMe, does not show any intramolecular hydrogen-bonding. A similar inverse turn structure of ΔAla was observed in the conformational studies of nisin (199).

In addition to restricting the peptide backbone, the dehydrostructure fixes the side chain conformation. The side chain conformation is one of the most important factors in generating a favorable interaction between peptides and acceptor molecules (receptors or enzymes). The Newman projections about the $C^{\alpha}-C^{\beta}$ bond in three classical low energy conformations of β-substituted amino acids in a peptide are depicted in Fig. 20.25. When these conformers of an L-amino acid are examined by standard steric factors, the gauche$^-$ (g^-; $\chi_1 = -60°$) and trans (t; $\chi_1 = 180°$) conformers show similar stability; the gauche$^+$ (g^+; $\chi_1 = 60°$) is the least stable. In the $C^{\alpha}-C^{\beta}$ dehydro structure, only two forms (Z and E: Fig. 20.23) are possible. The χ_1 of the Z-isomer is 0°, whereas that of E-isomer is fixed at 180°.

Because of the difficulty involved in the syntheses of (E)-dehydroamino acids (200, 201), the (Z) isomers have been studied more extensively. The dehydroamino acids have been incorporated into various bioactive peptides including peptide opioids (202–207), thyroliberin (208), TRH (209), bradykinin (210) and inhibitors of the N-acetylated α-linked acidic dipeptidase (211).

3.7 β,β-Dimethyl and β-Methyl Amino Acids

Side-chain dimethyl substitution strongly affects the conformation of a given residue. The global topology and dynamic prop-

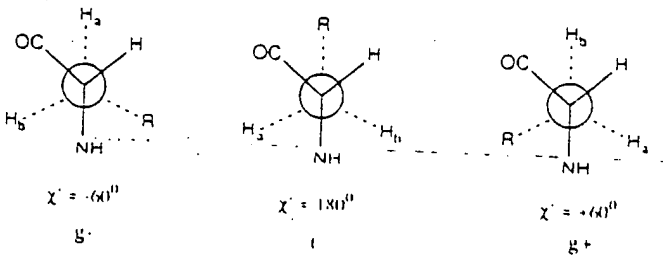


Fig. 20.25 Newman projections about $C^{\alpha}-C^{\beta}$ bond of β-substituted amino acids

erties of cyclic peptides are influenced by the bulkiness of geminal methyl groups. A β,β-dimethyl amino acid, penicillamine (Pen, β,β-dimethylcysteine) has been used for many disulfide bridged cyclic peptides including angiotensin II (212), oxytocin antagonists (213), peptide opioids (214, 215), RGD peptides (44), and others (216, 217). The incorporation of Pen affects the disulfide bond angles through steric constraints. When this amino acid was incorporated into cyclic enkephalins [i.e., Tyr-c(D-Pen-Gly-Phe-D-Pen)-OH; DPDPE], the resulting peptides were highly δ opioid receptor selective (Table 20.1) (218). These results indicate that the Pen can stabilize the conformation responsible for recognition at the δ-receptor. However, these peptides were less active than Tyr-c(D-Cys-Gly-Phe-D-Cys)-OH (218). It is possible that the extra methyl groups sterically hinder the interaction of the peptide with its receptor.

Monomethylation on the β-carbon (219) of amino acids can bias sidechain conformations by virtue of steric interactions. A β-methylated-β-substituted amino acid can have four different stereochemical structures because it contains two chiral carbons (2*S*,3*R*, 2*S*,3*S*, 2*R*,3*S* and 2*R*,3*R*). Each isomer favorably adopts one of the three different side-chain conformations (g^-, *t* and g^+) as mentioned in the previous section (Fig. 20.25). The 2*S*,3*R* isomer favors the g^- conformation and the 2*S*,3*S* isomer favors the *trans* conformation (Fig. 20.26). In addition, the 2*R*,3*S* isomer stabilizes the g^+ conformation while the 2*R*,3*R* isomer prefers the *trans* conformation.

This modification has been applied to the aromatic residues of a somatostatin analog, [c(Pro-Phe-D-Trp-Lys-Thr-Phe)] (220). The population of three side-chain conformations (g^-, *t* and g^+) was calculated from NMR studies (221). As speculated in Figure 20.26, the 2*S*,3*R*- and 2*R*,3*S*-aromatic residues contain a higher population of g^- and g^+, respectively. The 2*S*,3*S*- and 2*R*,3*R*-residues adopt a *t* conformation with increased populations compared to those of the parent analogs. The resulting analogs have shown different bioactivities, which were clearly dependent on the configuration at the α- and β-carbons. From the conformational analyses of these analogs, a model responsible for the bioactivity of somatostatin can be suggested. In addition, molecular dynamics calculations of these analogs permit the proposal of a pharmacophoric array which is dependent on the configuration of β-methyl groups.

Hruby et al. incorporated (βMe)Tyr and (βMe)Phe into positions 1 and 4 of DPDPE, respectively (222, 223). The resulting (βMe)Tyr-containing analogs were completely inactive at the opioid receptors. Among the analogs containing four isomers of (βMe)Phe, only the L-(βMe)Phe containing analogs were active but their activities were lower than those of DPDPE. The preference of L-amino acid at position 4 was also observed in the structure-bioactivity relationship study on their parent analogs. A possible explanation for the lower activities is the unfavorable interaction between these analogs and opioid receptors caused by steric hinderance due to the methyl group. Recently, 3,2'-dimethylphenylalanine was synthesized. This residue contains an additional methyl group on the 2 position of phenyl ring. When the stereo isomers of this residue were incorporated into oxytocin, all of the resulting analogs were active (224).

3.8 β-Substituted-2,3-Methano Amino Acids

These amino acids contain the structure of β-substituted Ac^3c (Fig. 20.27). They restrict the rotation of the N"–C" and C"–C(O) bonds in a manner similar to Ac^3c. The calculated global energy minimum of

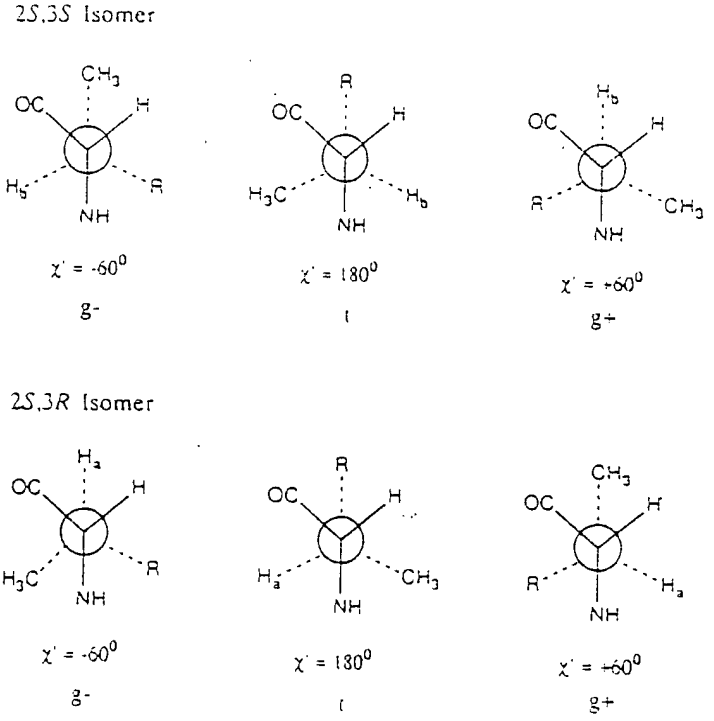


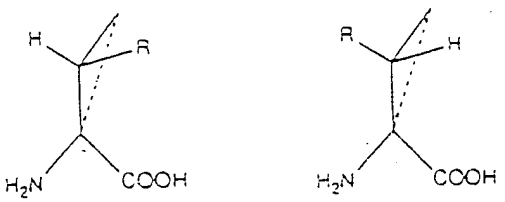
Fig. 20.26 Newman projections of the side chain conformations of (2*S*,3*S*)- and (2*S*,3*R*)-β-methylated-β-substituted amino acids.

the (1*S*,2*S*)-(*E*)-β-methyl-α,β-methanoalanine [or (1*S*,2*S*)-(*E*)-1-amino-2-methylcyclopropane carboxylic acid; (βMe)Ac^{3}c] was found to occur at $\phi = -90°$ and $\psi = 90°$ (225). Another minimum energy well was found to occur at $\phi = 90°$, $\psi = -60°$. The allowed ϕ, ψ conformational map of (1*S*,2*R*)-(*Z*)-(βMe)Ac^{3}c was also calculated. The results indicated that the minimum energy occurs at $\phi = -90°$ and $\psi = 90°$ (225).

Since (βMe)Ac^{3}c has two chiral carbons, four stereo isomers of this amino acid are available. All of these stereo isomers have been incorporated into taste ligands leading to Asp-(βMe)Ac^{3}c-OMe (226). The 1*R*,2*R* isomer containing compound is sweet, whereas the other analogs containing the other stereo isomers are tasteless. In addition, dimethyl and trimethyl Ac^{3}c were also synthesized and incorporated into taste ligands (226). Conformational analyses were carried out for all of the above taste ligands using ^{1}H-NMR and molecular modeling techniques (226). Their taste properties were explained on the basis of a previously reported topochemical model for taste response (227–231).

These 2,3-methanoamino acids severely restrict the rotation of $C''-C^{\beta}$ (χ_1) and $C^{\beta}-C^{\gamma}$ (χ_2). The χ_1 of the (*Z*)-isomers are fixed at +120° or −120° while those of the (*E*)-isomers are restricted to 0° (Fig. 20.27). The X-ray crystallographic study of the dipeptide, (*Z*)-(+)-(βPh)Ac^{3}c-Leu-OH [(βPh)Ac^{3}c denotes 1-amino-2-phenylcyclopropane carboxylic acid (or 2,3-methanophenylalanine)], indicates that the (βPh)Ac^{3}c residue adopts the conformation where $\phi = 88.4°$, $\psi = 21.5°$, $\chi_1 = -132°$ and $\chi_2 = 99°$ (232). The χ_2 angle of this structure can be explained by the ability of cyclopropane to restrict side-chain rotation.

These 2,3-methano amino acids have



E Z

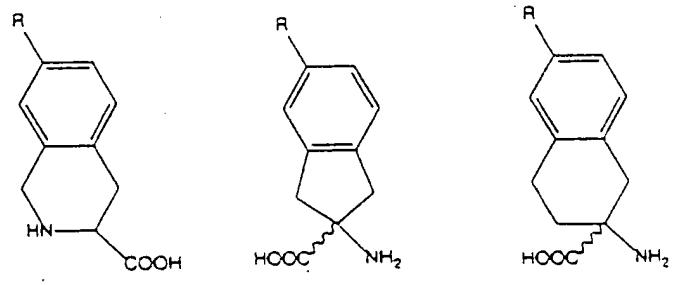
Fig. 20.27 Structures of *E*- and *Z*-isomers of β-substituted-2,3-methano amino acids.

also been incorporated into bioactive peptides. For example, four possible stereoisomers of (βPh)Ac^3c and (βHO-Ph)Ac^3c [1-amino-2-(4-hydroxy) phenylcyclopropane carboxylic acid or 2,3-methanotyrosine] were incorporated into the (D-Ala^2, Leu^5)-enkephalin sequence. Only one of the (*Z*)-(βPh)Ac^3c containing analogs showed bioactivity at the μ and δ opioid receptors while the others were inactive. Interestingly, among the inactive analogs, one of the (*E*)-(βPh)Ac^3c containing analogs exhibited antagonistic activity at the δ-receptor (233, 234). Most of the (βHO-Ph)Ac^3c (235) containing analogs were active only at the δ-receptor without any antagonistic activity (236).

Other 2,3-methano amino acids have been synthesized. These amino acids include 2,3-methanomethionine (237), 2,3-methanohomoserine (238) and others (239, 240). In addition, 3,4-methano amino acids such as 3,4-methanoglutamic acid (241, 242) and 3,4-methanohomophenylalanine (243) have been reported.

3.9 N–C^δ and C^α–C^δ Cyclized Aromatic Amino Acids

The cyclization between N and C^δ or between C^α and C^δ of Phe and Tyr has led to highly constrained amino acids such as 1,2,3,4 -tetrahydroisoquinoline -3-carboxylic acid (Tic) (98, 244, 245) 2-aminoindane -2-carboxylic acid (Aic) (98) 1-aminotetralin-2-carboxylic acid (Atc) (98), 7-hydroxy-1,2,3,4-tetrahydroisoquinoline carboxylic acid (HO-Tic) (246, 247), 2-amino-5-hydroxyindane carboxylic acid (Hai) (248), and 2-amino-6-hydroxytetralin-2-carboxylic acid (Hat) (Fig. 20.28) (248, 249). These amino acids are particularly effective for fixing the rotation around C^α–C^β (χ_1) and C^β–C^γ (χ_2) bonds. The Tic residue restricts χ_1 to either −60° (g^-) or 60° (g^+). The $\chi_1 = 180°$ (t) side-chain rotamer is excluded. The χ_2 for this residue is about 160° (98, 244, 235). In the Aic or Hai residues (Fig. 20.28B), χ_1 and χ_2 are restricted to −80 and −20° or −160 and 20°



A B C

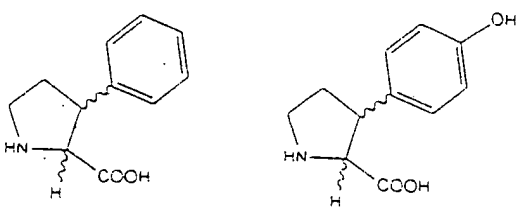
Fig. 20.28 Structures of C^α–N^α and C^α–C^α cyclized amino acids. A, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic, R = H) and 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (HO-Tic, R = OH); B, 2-aminoindane-2-carboxylic acid (Aic, R = H) and 2-amino-5-hydroxyindane-2-carboxylic acid (Hai, R = OH); and C, 2-aminotetralin-2-carboxylic acid (Atc, R = H) and 2-amino-6-hydroxytetralin-2-carboxylic acid (R = OH).

(98). The Atc and Hat residues (Fig. 20.28C) can adopt only two side-chain conformations. These conformations are characterized by torsion angles [$\chi_1 = 180°$ (t), $\chi_2 = 25°$] or [$\chi_1 = -60°$ (g^-), $\chi_2 = -25°$] for the L-residue and [$\chi_1 = 180°$ (t), $\chi_2 = -25°$] or [$\chi_1 = 60°$ (g^+), $\chi_2 = 25°$] for the D-residue (98).

The Tic, Aic, and Atc compounds were incorporated into a dermorphin analog Tyr-c(D-Orn-Phe-Glu)-NH_2 as modifications of Phe by Schiller et al. (98). The resulting analogs, which contained Aic, D-Atc and L-Atc showed bioactivities at the receptor binding assays, whereas the Tic containing analog was totally inactive. Hruby et al. incorporated the Tic residue into μ opioid receptor selective antagonists to examine the side-chain conformations for bioactivity (250). A new series of δ opioid receptor selective antagonists have been obtained using the Tic residue by Schiller et al. (251). The Tic residue has also been incorporated into bradykinin (161). When the Hai and Hat were incorporated into positions 1 and 4 of the enkephalin sequence, respectively, the resulting analog with Hat showed μ-receptor selective activity, whereas the analog with Hai was inactive (248). In addition, the Aic^3-containing chemotactic peptide analog, HCO-Met-Leu-Aic-OMe, was highly active (252).

3.10 Substituted Proline

All three types of amino acids described in Section 3.9 are effective in the restriction of the conformations around the $C''-C^{\beta}$ bond because of the inclusion of this bond in a 5- or 6-membered ring. However, because of their bicyclic structures, these modifications also limit the allowed values of χ_2. Thus, β substituted prolines are attractive as mimetics since they allow for conformational freedom in the χ_2 rotation of the side chain. In this structure, the χ_1 rotation is limited in range from −85 to −150°.



A B

Fig. 20.29 Structures of A. β-phenylproline [(βPh)Pro] and B. β-(4-hydroxyphenyl)proline (Hpp).

The β-phenylproline [(βph)Pro: Fig. 20.29A (253)] and β-(4'-hydroxyphenyl)-proline (Hpp: Fig. 20.29B (254) residues were synthesized and incorporated into opioid peptides. These proline analogs can be considered as $N''-C^{\beta}$ cyclized phenylalanine or tyrosine. When the enantiomeric mixture of *trans*-β-phenylproline was incorporated into Tyr-Pro-X-$N(CH_3)_2$, only one of the resulting analogs was active (253). The *trans*-Hpp containing cyclic dermorphin analog *t*-Hpp-c(D-Cys-Phe-D-Pen)-OH showed activity similar to that of the parent analog, whereas the D-*t*-Hpp containing analog was completely inactive (254).

Other functional groups, for example, the propyl and carboxyl groups, have been incorporated into the β position of Pro (255). Various α and γ-substituted prolines were also synthesized and are likely candidates to be incorporated into bioactive peptides (256–258).

3.11 Miscellaneous Mimetics for Amino Acids

The incorporation of a bulky side chain can provide conformational constraints for a peptide. The bulky groups can restrict the movements of the other side chains of the peptide. This steric interaction may lead to changes in the conformation of peptide

backbones. Naphthylalanine [βNal(1) or βNal(2)] (259, 260) and *O*-*t*-butylserine or *O*-*t*-butylthreonine (261, 262) were incorporated into cyclic and linear enkephalins. The resulting analogs were more constrained and showed higher bioactivities than the parent analogs (260, 263).

The incorporation of a D-amino acid is one of the most popular modifications. In naturally occurring peptides, D-amino acids are often observed as a critical residue for bioactivity. For example, the D-amino acids at position 2 of dermorphin and deltorphins are required for opioid activities (218). In many cases, the incorporation of a D-amino acid increases bioactivities of peptides including enkephalins (218), somatostatin (31), and oxytocin (264). When the second residue of enkephalin (Gly) was replaced with D-Ala, the resulting analog showed a higher bioactivity and metabolic stability than the parent. The replacement with L-Ala generated an inactive analog. In addition, incorporation of the D-residue can affect the secondary structure of peptides. If the central residues of a β-turn ($i + 1$ and $i + 2$ residues) have the L-configuration, a type I turn is often formed. If the residue at the $i + 1$ position is L and the residue at position $i + 2$ is D (an L,D-pair), then a type II form is stabilized. A D,L-pair at the central position will stabilize a type II′ turn. These results are well summarized in a review by Rose et al. (185).

Combinations of amino acid modifications have also been considered. The (NMe)Aib and (NMe)Ac^{5}c have been synthesized in which *N*-methylation and α-alkylation are combined. An attempt was made to incorporate these residues in place of Pro^{11} of the cyclic somatostatin analog [c(Pro^{11}-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11})] (265, 266). After the partially protected linear hexapeptides H-D-Trp-Lys(Boc)-Thr(*t*Bu)-Phe-(NMe)Aib / (NMe)Ac^{5}c-Phe-OH were assembled, they were cyclized. During the removal of the Boc and *t*Bu protecting groups in the presence of trifluoroacetic acid, half of the (NMe)Aib containing cyclic molecule was ring-opened. In case of the (NMe)Ac^{5}c structure, all of the protected cyclic peptide was ring-opened. The cleavage sites are identified as the amide bond of (NMe)Aib and (NMe)Ac^{5}c to the succeeding phenylalanyl residue by NMR spectroscopy. Preliminary results of conformational studies for the protected (NMe)Aib and (NMe)Ac^{5}c containing cyclic peptides indicated that the amide bond between (NMe)Aib/(NMe)Ac^{5}c and the succeeding residue Phe is highly constrained.

The β-amino-tetrahydronaphthyl carboxylic acid (βAtnc) has also been synthesized as a constrained Phe analog on the basis of the β-amino acid structure (120). The α-benzylproline is a new type of constrained amino acid which incorporates the N–C$^\alpha$ cyclization on the Phe structure (256).

4 MOLECULAR MIMICS OF PEPTIDE SECONDARY STRUCTURES

The secondary structures of most peptides and proteins show well-defined conformational features. It is well known that these structures are critical for the bioactivities of peptides. The secondary structures of peptides can be interchanged because of inherent flexibility. Thus, efforts have been made to fix specific secondary structures in peptides by use of peptidomimetic structures. A recent issue of *Tetrahedron* was devoted to the studies of peptidomimetics for peptide secondary structures (267).

One of the most important structural features of peptides and proteins is the β-turn. In many proteins, β-turn structures are exposed and may be part of ligand recognition sites (185, 268). Furthermore, β-turns are common conformations for many bioactive peptides. In Figure 20.30, representative nonpeptidic β-turn mimics are depicted. When these structures were

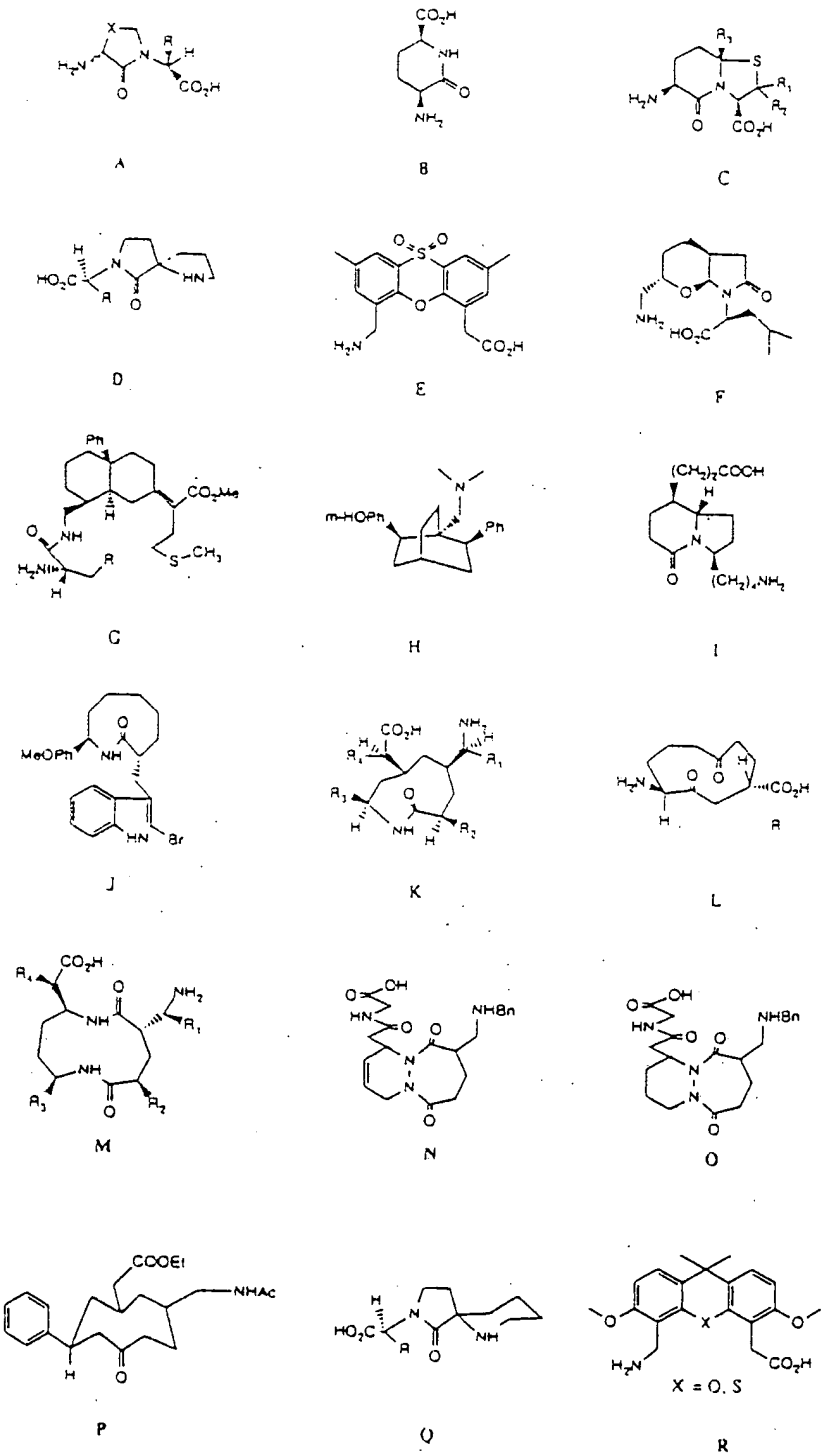


Fig. 20.30 Structures of β-turn mimics. The structure C was originally proposed but the modeling studies indicated that the structure with reversed configuration of the carbon substituted by R_1 is closer to the β-turn. Personal communication with Dr. Klaus Muller

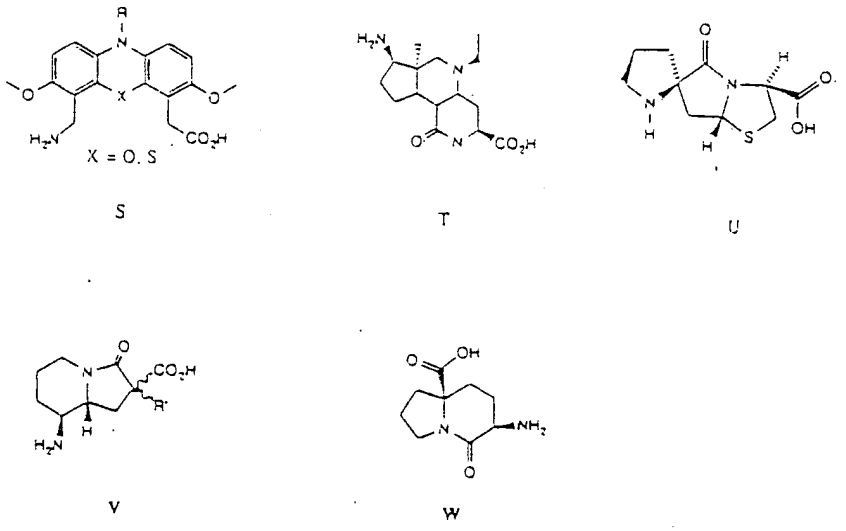


Fig. 20.30 (*Continued*)

incorporated into bioactive peptides, some of the resulting analogs showed high activity. The studies on the nonpeptidic β-turn mimics were reviewed by Ball and Alewood (Fig. 20.31A–M) (269), and their modified forms or new β-turn mimics were reported (Fig. 20.31N–W) (270–279).

A γ-turn mimic has been synthesized by Huffman et al. (280). In the Figure 20.31, the generalized γ-turn (A) and Huffman's mimic (B) are depicted. Compared to an idealized γ-turn, where torsional angles are $\psi_i = 120°$, $\phi_{i+1} = 80°$, $\psi_{i+1} = -65°$, and $\phi_{i+2} = -120$, the corresponding angles determined by X-ray crystallography of the mimic are $\psi_i = 128°$, $\phi_{i+1} = 56°$, $\psi_{i+1} = -67°$ and $\phi_{i+2} = -123°$ (280). When this mimic was incorporated into RGD antagonists, the resulting analogs were highly active but, interestingly, enkephalin analogs were inactive (281). It may be that γ-turn destroys the enkephalin recognition at the receptor. Kahn et al. synthesized another γ-turn mimic (Fig. 20.31C). When this structure was incorporated into bradykinin, the resulting analog exhibited bioactivity (282). Kemp and Carter also suggested two structures as γ-turn templates (Fig. 20.32) (283).

In addition, mimics or templates of β-sheet structure have been studied. In the conformational studies of a naturally occurring antitumor agent bouvardin, it is indicated that the β-sheet structure of this

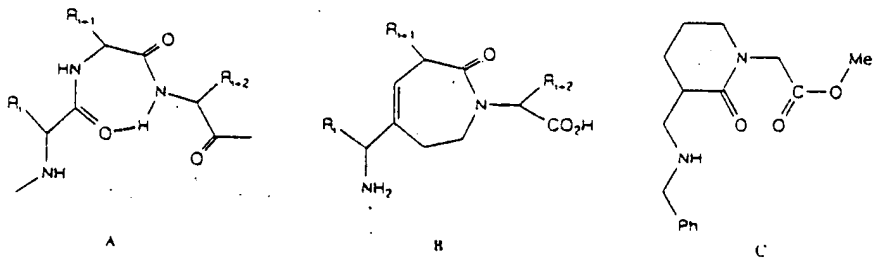


Fig. 20.31 Typical γ-turn structure; and B, C, its mimetic structures.

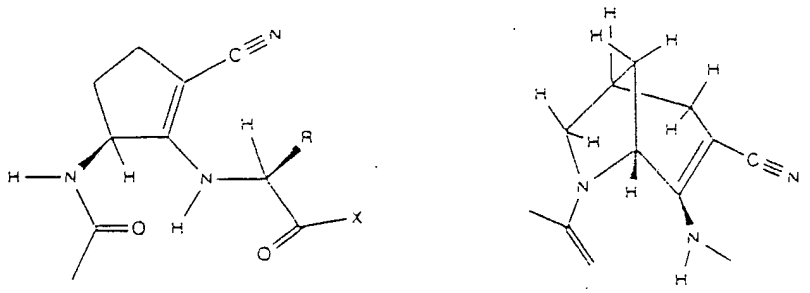
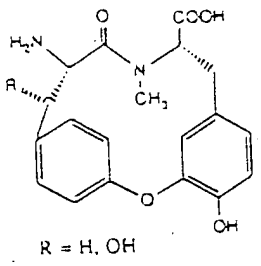


Fig. 20.32 Structures of γ-turn templates.

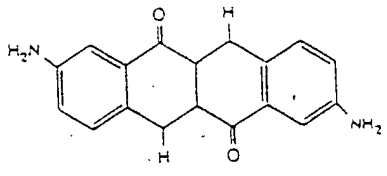
compound can be stabilized by the structure depicted in Figure 20.33A (284). Kemp et al. mimicked and induced β-sheet structure by using epindolidione derivatives (Fig. 20.33B) (285, 286). When this structure was incorporated into a peptide, the NMR parameters (temperature coefficients for amide proton, coupling constant for α- and amide proton and NOEs measured in DMSO) of the peptide indicated that the resulting peptide adopted β-sheet structure. The compound 2'-aminomethylbiphenyl-2-carboxylic acid (Fig. 20.33C) was devised for the same purpose (287). However, it was found to generate a conformation between the geometries of an idealized β-sheet and γ-loop (287). Recently, two new structures which can stabilize a β-strand were reported by Diaz et al. (Fig. 20.34D) (288) and by Smith et al. (Fig. 20.34E) (289). In the study by Diaz et al. (288) 4-(2-aminoethyl)-6-dibenzofuranpropionic acid (Fig. 20.34D) was incorporated in place of D-Phe-Pro in the linear gramicidin S octapeptide analog Val-Lys-Leu-D-Phe-Pro-Val-Lys-Leu-NH_2. It was also incorporated into a larger related peptide, Lys-Val-Lys-Val-Lys-Val-[4-(2-aminoethyl)-6 -dibenzofuranpropionic acid]-Val-Lys-Val-Lys-Val-Lys-NH_2. The NMR and CD studies of these peptides in aqueous solution indicated that the mimetic (Fig. 20.33D) can stabilize the antiparallel β-sheet structure. The mimic reported by Smith et al. (Fig. 20.33E) is characterized by the pyrrolin-4-ones (289). The X-ray analysis of a model compound including this structure (Fig. 20.33E) indicated the presence of an antiparallel β-pleated-sheet structure (289).

Helical structure can also be stabilized by peptidomimetic structures. Kemp et al. synthesized mimetic structures in which the pyrrolidine rings of two consecutive Pro residues were connected by a thiomethylene. The resulting units could act as templates nucleating helical conformations (290, 291). Muller et al. incorporated derivatives of diacyl-azabicyclo[2.2.2]octane (Fig. 20.34A) and Kemp's triacid (Fig. 20.34B) into the *N*-terminus of peptides as templates for α-helical structure. The resulting peptides exhibited substantially increased helicity compared to the peptides without such templates (275).

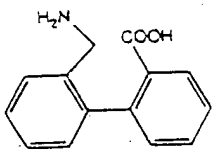
In addition, dipeptide structures can be designed as constraints to restrict the conformational flexibilities of the backbone and side chains. The resulting units display unique conformational preference and can thus stabilize the secondary structures of peptides. Toniolo has reviewed these types of constrained units extensively (128). Also, several subsequent reports on the same structures described in the review article (128) have been published (292–297). Recently, new types of constrained dipeptide units have also been reported (298, 299).



A



B



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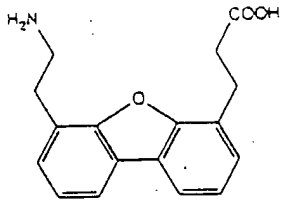

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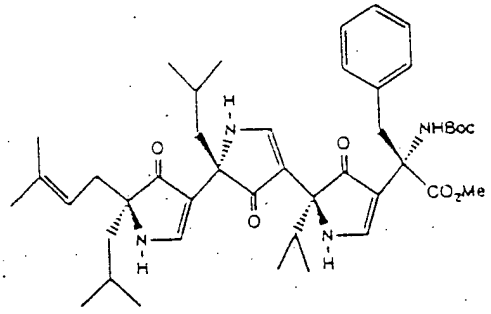
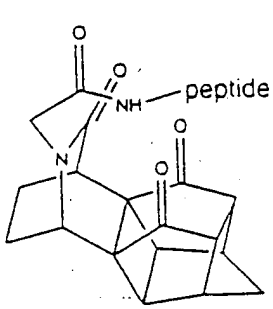
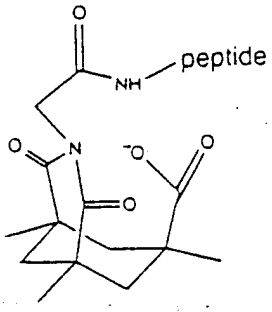


E

Fig. 20.33 Mimics of secondary structures.



A



B

Fig. 20.34 Derivatives of A. diacyl-azabicyclo[2.2.2]octane; and B. Kemp's triacid as templates for α-helical structure

5 AMIDE BOND ISOSTERES

Peptides are rapidly and specifically degraded by enzymes in biological systems. For example, the (Leu^5)-enkephalin in a rat-brain homogenate degraded in a matter of minutes. To increase metabolic stability of biologically active peptides, many amide bond isosteres have been devised. These peptide bond surrogates resemble the amide bond but are more resistant to enzymatic cleavage. Most of these modifications are accompanied by changes in geometric or topochemical structure, electronic distributions, and hydrophilic or lipophilic properties. The introduction of amide isosteres results in local and global changes in dipole moments and in the pattern of intramolecular as well as peptide-receptor hydrogen-bond formation. Thus, peptide bond modifications not only increase metabolic stability but can improve selectivity towards the receptor subtypes, change pharmacological functions and enhance pharmacokinetic properties such as oral bioavailability, transportability across the blood–brain barrier and duration of action at the target tissues.

As in the case of constrained amino acids, isosteric modifications at selected sites of a peptide sequence can act as conformational probes by allowing different degrees of rotational freedom in the backbone. Selected properties of the amide bond can be removed through the peptide backbone modifications, which may allow the examination of the specific requirements for bioactivity. These characteristics make the amide bond isosteres attractive tools for studying bioactive peptides and designing new drug molecules.

5.1 The Retro-Inverso Modifications [NH–C(O)]

As a means of protection against enzymatic cleavage as well as preservation of amide geometry, the retro–inverso modification has been one of the most widely used amide isosteres (300, 301). In this modification, specific peptide bonds are reversed in direction resulting in a *gem*-diaminoalkyl residue (*g*AA) on the amino-terminal end and a 2-alkylmalonyl residue (*m*AA) on the carboxyl-terminal end of each reversal (Fig. 20.35). This one-bond reversal is referred to as the "pairwise" retro–inverso modification because it affects pairs of adjacent residues. If the reversal operation is repeated for two or more successive amide bonds in a peptide, the modification is referred to as an "extended" retro–inverso modification. The sequence-direction of central residue(s) in the altered segment should be reversed to give retro-amino acids (rAA: Fig. 20.35). These modifications have been incorporated into numerous bioactive peptides. Many of the resulting analogs are highly active, selective, and metabolically stable. Recently these results have been reviewed by Chorev and Goodman (302) and the review also includes the development of synthetic methods and numerous conformational studies of these modifications.

In addition, the retro–inverso modification is useful for designing new cyclic structures. For example, cyclization by connecting two carboxylic groups is facilitated by using the *gem*-diaminoalkyl residue. To connect two amine groups, dicarboxylic acids are the most appropriate. These concepts were applied for cyclizing bioactive peptides including opioid peptides (303) and others (304).

5.2 The Reduced Amide Bond (Methyleneamine: CH_2–NH)

Methyleneamine is a reduced form of amide bond. This amide isostere ($-CH_2-NH-$) does not have the double bond character. Thus, the reduced peptide bond offers free rotation around the C–N bond

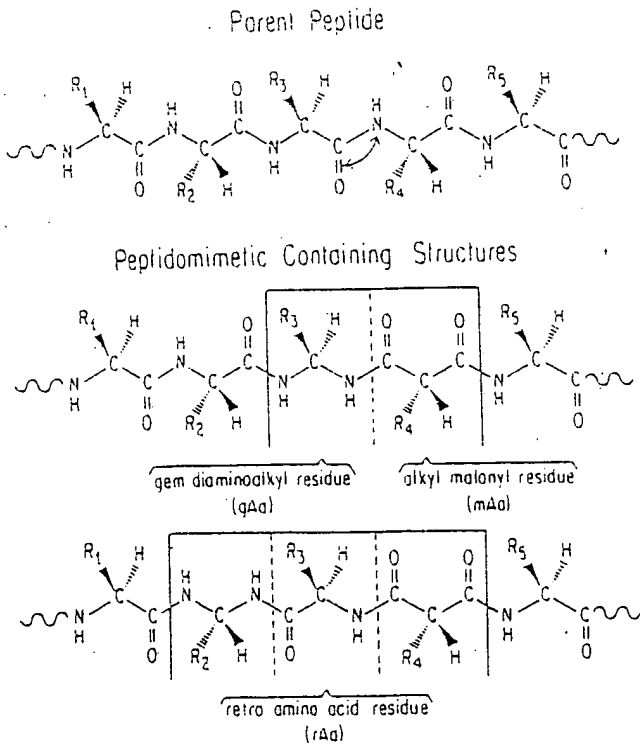


Fig. 20.35 Structures of retro-inverso modifications.

(ω angle). In addition, since the amino group of methyleneamine is protonated at physiological pH, the methylene cannot act as a H-bonding acceptor.

The influence of a protonated methyleneamine bond on the secondary structure of peptides was studied by Marraud et al. (166, 305, 306) using the reduced bond modified dipeptides, *t*BuC(O)-Pro-Glyψ-[CH_2-N^+H_2Et]$^+B^-Ph_4$ and *t*BuC(O)-Pro-Glyψ[CH_2-N^+H_2Me]$^+B^-Ph_4$ (where ψ denotes a pseudopeptide or surrogate bond), the effects of this modification on the ability to form a β-turn were studied. In solution, IR and ^{1}H-NMR experiments showed evidence of strong hydrogen-bonding between N^+–H and C(O) of the *t*-butylcarbonyl groups. The protonation of an amine in the reduced bond facilitated the formation of a 10-membered ring, similar in structure to β-turns. This result indicated that a reduced amide bond in pseudopeptide analogs can retain β-folding tendencies at physiological pH.

When the amine of the reduced amide bond is not protonated, the overall conformation of the pseudopeptide unit can be very different. Van Binst et al. studied Pro-Leu-Gly-NH_2, which is potentially useful in the treatment of mental depression and Parkinsonism (307). This peptide adopts a preferred C_{10} β-turn conformation in DMSO or acetonitrile and in the crystalline state. When an amide bond is replaced by a methyleneamine, the conformation of the resulting peptides shows a dependence on protonation status. The HCl$^+$Pro-Leuψ[CH_2-NH]Gly-NH_2 adopts a similar conformation to the parent whereas the HCl$^+$ Pro-Leuψ[CH_2-NH^+HCl]Gly-NH_2 does not display a β-turn structure.

When one of the amide bonds in bombesin (308–310), secretin (311), peptide opioids (312–316), substance P (317, 318),

gastrin (319), and growth-hormone releasing factor (320) is replaced by this modification, the resulting peptides show either agonistic or antagonistic activities. The reduction of an amide bond in a peptide altered the pharmacological role from agonist to antagonist as well as changed agonistic potencies. Bioactivities (either agonistic or antagonistic) were found to be dependent on the position of the modification. This modification has also been incorporated into other bioactive peptides including cholecystokinin (321, 322), oxytocin (323), somatostatin (324), human renin inhibitors (325, 326) and HIV protease inhibitors (327) leading to active analogs. Spectroscopic studies of several peptides with this modification (morphiceptin (313), gastrin (328) and bombesin (329) have also been reported.

Since the reduced amide bond is relatively flexible compared to the amide, the introduction of constraints has been investigated. Examples include alkylmethyleneamine [CH(R)–NH; Fig. 20.36A] (330), amidomethyleneamine (CH[C(O)–NH_2]–NH; Fig. 20.36B (331), methylene-*N*-acetylamine [CH_2–N(Ac); Fig. 20.36C] (332, 333), methylene-*N*-formylamine [CH_2–N(For); Fig. 20.37] (332) and methylenealkylamine [CH_2–N(R); Fig. 20.36D (334). The methylenealkylamine modified neurokinin analogs show bioactivities similar to or higher than those of amide- or methyleneamine-containing analogs (334). When amidomethyleneamine is incorporated into the angiotensin II analog, the resulting peptide is inactive (331).

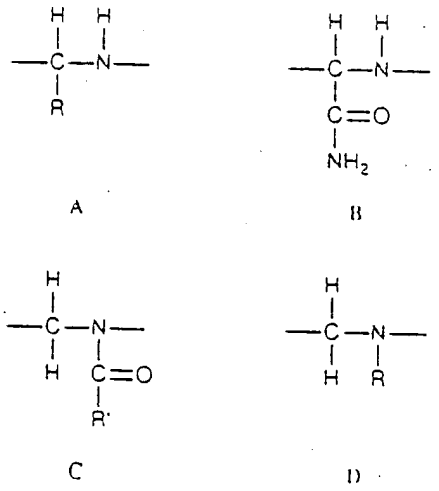


Fig. 20.36 Structures of modified reduced amide bonds. A, –CHR–NH–; B, –CH[C(O)–NH_2]–NH–; C, –CH_2–N[C(O)–R']–; and D, –CH_2–NR–.

5.3 Methylenethioether [CH_2–S] and Methylenesulfoxide [CH_2–S(O)]

The methylenethioether modification was considered as an amide isostere which could offer polarity, flexibility, and metabolic stability. Spatola et al. incorporated this modification into LH–RH (335) and peptide opioids (315, 336–339). The stability of the methylenethioether modified analog in biological systems was examined using a linear enkephalin analog, Tyr-Gly-Gly-Pheψ[CH_2–S]Leu-OH (340). This analog showed a 21-fold longer half-life than leucine enkephalin when it was subjected to blood serum. Incorporation of this modification has generated highly active analogs. For example analogs with this modification, Tyr-D-Ala-Gly-Pheψ[CH_2–S]Leu - NH_2 and Tyr - c[D - Lys - Gly - Pheψ [CH_2–S]Leu], show higher activity at both μ and δ opioid receptors than their corresponding all-amide parent peptides (315, 337). When this modification was introduced into a somatostatin analog, the activity of the resulting c[Proψ[CH_2–S]Phe-D-Trp-Lys-Thr-Phe] exceeded somatostatin tetradecapeptide (341). However, it is only six percent of the activity of their parent analog c(Pro-Phe-D-Trp-Lys-Thr-Phe). An LH-RH analog, [$Gly^6\psi$[CH_2S]-Leu^7]LH-RH, displayed low potency when compared to its all amide bond counterpart, possibly because the CH_2S moiety imparted increased flexibility at the β-turn centered at 6–7 position (335). In addition,

these modifications have been incorporated into oxytocin (323), CCK-B dipeptide antagonists (333), and a renin inhibitor (326).

Molecular modeling studies suggest that the methylenethioether modification is compatible with secondary structures of peptides and proteins. To investigate this point, Spatola et al. studied c(Gly^1-Pro^2-Gly^3-D-Phe^4-Pro^5) and its methylenethioether modified analogs using NMR spectroscopy (342, 343). The parent peptide contains a β-turn and a γ-turn through a hydrogen bond between Gly^{1}C(O) and D-Phe^{4}NH and another hydrogen bond between D-Phe^{4}C(O) and Gly^{1}NH (344–347). A single conformer whose amide bonds are all in a trans configuration is observed in $CDCl_3$. However in DMSO, this peptide displays 10% of a second conformer in which a cis amide bond is included. A methylenethioether modified model compound, c(Gly-Proψ[CH_2–S]Gly-D-Phe-Pro), can adopt both β- and γ-turns in $CDCl_3$, as assessed by chemical shift data temperature coefficients and solvent dependence data. In DMSO, the ratio of the two isomers is 3:2. The major conformer contains a cis amide bond at the Gly-Pro bond, but retains the intramolecular γ-turn hydrogen bond (343). Another pseudopeptide, c(Proψ[CH_2-S]-Gly-Pro-Gly-D-Phe), shows similar phenomena in both solvents. The population of the all trans conformer in DMSO is 55%. This result is interesting because the pseudopeptide retains the same overall conformation as its parents though the C(O) for the γ-turn hydrogen bond was replaced with methylene (342). In summary, the model peptide and its analogs modified with methylenethioether can adopt the same conformation as the parent peptide. In the above case, steric restrictions on the backbone are retained upon replacement of an amide bond by a methylenethioether surrogate group.

The thioether is easily oxidized to generate *R*- and *S*-sulfoxide. The resulting methylenesulfoxide is also a useful amide bond isostere because of the fact that it contains an additional stereogenic center, a highly constrained structure and relatively strong hydrogen bond-accepting capacity (as compared with the methylenethioether). Thus, Spatola et al. studied c(Gly-Pro-ψ[CH_2-(*R*/*S*)-S(O)]-Gly-D-Phe-Pro) and compared the results with the studies of its all-amide parent and methylenethioether-modified precursor (348). The NMR studies indicate that the methylene sulfoxide-modified pseudopeptide induces conformational changes that are distinctly different from its parent and precursor molecules. When this modification was incorporated into cyclic enkephalin, both isomers of the resulting analog, Tyr-c(D-Lys-Gly-Pheψ[CH_2-(*R*/*S*)-S(O)]Leu), have shown similar bioactivities to the all-amide parent analog and methylenethioether-modified precursors (337).

5.4 Methylene Ether (CH_2–O)

The methylene ether bond (–CH_2–O–) (349, 350) has some advantages over the methylenethioether. The nucleophilicity and oxidation possibility of oxygen are negligible compared with sulfur. The ether has higher polarity and can form stronger hydrogen bonds. Furthermore, the CH_2–O- group possesses closer geometric resemblance to an amide than the methylenethioether linkage (351). Chorev et al. introduced this modification into substance P and Leu-enkephalinamide (352). The resulting *p*Glu-Phe-Pheψ[CH_2–O] Gly-Leu-Met-NH_2 retains the activity of the parent analog. The Tyrψ[CH_2–O]Gly-Gly-Phe-Leu-NH_2 is twice as active as its all-amide parent analog at the μ opioid receptor and shows reduced activity in the δ opioid receptor. This modification has also been incorporated into a renin inhibitor (326, 353), and the CCK-B dipeptide antagonist (333) to produce active analogs.

5.5 Ethylene ("Carba": CH_2–CH_2)

Although the carba replacement (354–356) is a modification of the reduced amide bond (similar to methylene thioether or methylene ether), it possesses different characteristics because the whole "carba" surrogate is nonpolar. This modification does not allow for the possibility of intramolecular hydrogen-bonding. This characteristic leads to more flexible backbone conformations. When this modification is incorporated into Tyr-Gly of Met-enkephalinamide, it displays an order of magnitude decrease in activity (312). However, Tyr-Glyψ[CH_2–CH_2]Gly-Phe-Met-OH shows higher activity than Met-enkephalin in *in vivo* tests (357). A "carba" modified cholecystokinin shows activity similar to its all-amide parent molecule (321), while a renin inhibitor with this modification shows only minimum activity (326).

5.6 Thioamide [C(S)–NH]

With the introduction of facile thionating reagents such as Lawesson's reagent (358, 359), thioamides have been easily incorporated into peptides as amide isosteres. Spectroscopic and X-ray studies of di- and trithiopeptides, using various spectroscopic techniques, revealed that a thioamide adopts a *Z* planar configuration similar to that of an amide (360–365). The bond length of the thiocarbonyl (1.64 Å) and the covalent radius of sulfur (1.04 Å) are much longer than those of carbonyl and oxygen (1.24 Å and 0.74 Å, respectively) (366). Thus, the larger volume of the thioamide can restrict the allowed torsional angles in the vicinity of the thioamide more than in that of the amide. The computational studies of the thioamide bond containing small peptides have indicated that the allowed ϕ, ψ conformational space for thioamide containing residue is reduced (367, 368). However, the regions where experimentally most protein conformational angles are observed are not drastically affected except in the area with high ψ values. Studies of the hydrogen-bonding properties of thioamides have shown that the NH of a thioamide is a stronger H-bond donor (higher acidity) and the thiocarbonyl is a weaker H-bond acceptor than the corresponding amide (369). Thus, hydrogen-bonding between C(O) and HN–C(S) is stronger while the H-bonding between C(S) and HN–C(O) is weaker as compared to the hydrogen-bond between C(O) and HN–C(O) (360–363, 370). The incorporation of the thioamide isostere in key positions of peptide analogs can lead to compounds with either enhanced or reduced conformational flexibility depending on whether steric or hydrogen bonding forces prevail.

Spatola and coworkers carried out studies to examine the compatibility of a thioamide with reverse turn features using the specific sequences of model peptides (371) as they did in the studies of methylenethioether (342, 343) and methylenesulfoxide (348). The NMR studies of the model compound, c(Proψ[C(S)-NH]Gly-Pro-Gly-D-Phe), have shown that this molecule can adopt the same general conformation (in $CDCl_3$ and DMSO) as its all-amide parent (344–347). The model compound c(Gly-Proψ[C(S)-NH]-Gly-D-Phe-Pro) retained the same conformations as the parent in $CDCl_3$ but exhibited two conformers in DMSO with a ratio 2:1. The minor conformation contains a cis amide bond at the Gly–Pro bond. The strength of the intramolecular hydrogen-bonding for β- and γ-turns, estimated from the temperature coefficients of an amide proton, are equal to and a little weaker than those of the parent molecule, respectively. Thus, the overall conformational effects of the thioamide modification may not be dramatic.

Many bioactive peptide analogs incor-

porating this modification have been synthesized. When thioamides were incorporated into oxytocin (372), the activity of the resulting analogs was not high. Thioamide analogs of the thyrotropin-releasing hormone (i.e., *p*Glu-His-Proψ[C(S)-NH]H) were synthesized and showed a similar potency to the parent analog (373–375). A C-terminal growth hormone releasing hexapeptide analog with this modification (His-D-Trp-Ala-D-Phe-Lysψ[C(S)-NH]H) was completely inactive (376). In analogs of Leu-enkephalin, modification of the amide bond between residue 1 and 2 produced an inactive compound, whereas modification of the amide bond between residue 2 and 3 led to an analog that was more potent and selective for the δ-receptor than Leu-enkephalin (377, 378). This modification was also incorporated into cyclic enkephalins (379), CCK-B dipeptide antagonists (333), peptide-substrates of carboxypeptidase A (380), substance P (381), gastrin (382), chemotactic peptide (383), bombesin (384) and leucine aminopeptidase (385). The resulting analogs were found to be more stable against enzymatic degradation than their amide counterparts. In addition, to examine the conformation responsible for the bioactivities, the X-ray studies of thioamide containing chemotactic peptides (286) and protected Leu-enkephalin were carried out (387).

5.7 *trans*-Olefin (CH=CH, *trans*) and *trans*-Fluoroolefin (CF=CH, *trans*)

The peptide bond in polypeptides and proteins generally assumes a trans configuration since its cis counterpart induces unfavorable steric interactions. Among the mimics of amide bonds which have been reported, the *trans* carbon–carbon double bond (olefin: Fig. 20.37A) is most suitable to mimic the linkage in terms of geometry, bond angle and bond length. Whereas the amide bond has some degree of flexibility

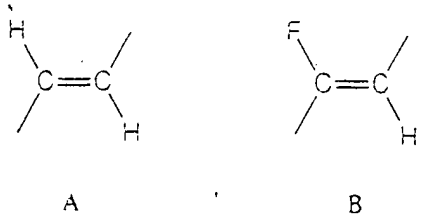


Fig. 20.37 Structures of A, *trans*-olefin; and B, *trans*-fluoroolefin.

and possesses hydrogen-bonding capability, the trans olefin fixes the replaced peptide linkage in a trans configuration and eliminates all possibility of hydrogen-bonding. This modification can provide valuable information concerning the role of an amide bond at a specific site in a peptide, in its bioactivity profiles and conformational behavior. Furthermore, the substitutions of an amide bond with a trans olefin increases the hydrophobicity of the resulting peptides and thus potentially facilitates biotransportability through cell membranes, including passage through the blood brain barrier. Improved metabolic stability is also observed. A number of synthetic methods for this modification have been developed and applied towards the synthesis of bioactive peptide analogs (388–392).

Dermorphin and its tetrapeptide analogs which incorporated this modification between Phe and Gly, showed activity comparable to the parent molecules (393). The conformational behavior of the trans olefin bond was examined using these analogs. All of the olefin modified analogs exhibited essentially the same ^{1}H-NMR parameters: chemical shifts, temperature coefficients for amide protons, and coupling constants between NH and C$^{\alpha}$H and between C$^{\alpha}$H and C$^{\beta}$H. The sets of NOE observed from dermorphin and Tyr-D-Ala-Pheψ (CH=CH, *trans*) Gly-Tyr-Pro-Ser-NH$_2$ are also the same in terms of their pattern and intensity. The incorporation of a trans olefin bond in place of the amide bond between residue 3 and 4 did not alter the conformational

characteristics of the peptides, in DMSO solution. These results are in agreement with those of the theoretical studies of Ac-Ala-NHMe, Ac-ψ[CH=CH, *trans*] Ala-NHMe, Ac-Alaψ[CH=CH, *trans*]-CH_3 and Ac-ψ[CH=CH; *trans*] Alaψ[CH=CH, *trans*]-CH_3 (394, 395). In these studies, a single substitution of the peptide bond with trans-olefin bond does not introduce dramatic changes in conformational preferences. This modification was incorporated into other bioactive peptides including linear and cyclic enkephalin analogs (396–398), substance P (397), angiotensin-converting enzyme inhibitors (399), renin inhibitors (326), inhibitors of protein kinase (389, 391, 400) and others (323, 333).

Recently, a fluoroolefinic isostere [ψ(CF=CH, *trans*): Fig. 20.37B] was designed and utilized (401). Because the electronic properties of fluorine are similar to those of oxygen, this modification seems to resemble amide more than the simple olefin. This modification has already been incorporated into the amide bond between Phe and Gly of the full length of substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Phe-Gly-Leu-Met-NH_2) and *p*Glu-Phe-Phe-Gly-Leu-Met-NH_2. The bioactivity of the fluoroolefin modified substance P analog with this modification is similar to that of the natural peptide. The *p*Glu-Phe-Pheψ[CF=CH, *trans*]-Leu-Met-NH_2 is ten times more active than the analog with a *trans* ethylene group. The ^1H, ^{13}C and ^{15}N chemical shifts, temperature coefficients, and coupling constants of substance P and its fluoroolefin modified analog are very similar. These results suggest an overall similarity between amide and fluoro olefin bonds. This amide isostere has also been incorporated into opioids leading to an active analog (402).

5.8 1,5-Disubstituted Tetrazole Ring (CN_4)

To mimic a cis amide bond, Hann et al. attempted to synthesize a cis-olefin. However, the resulting olefin quickly isomerized to a more stable trans-olefin (403). Thus, Marshall et al. proposed a tetrazole (404) ring as an amide isotere in order to lock an amide bond into a cis configuration (Fig. 20.38) (405). The possible conformations available to the linear dipeptide, Ac-Ala-Ala-NHMe, in which the central amide bond was fixed in the cis configuration was compared with Ac-Alaψ[CN_4]Ala-NHMe, in which the central amide bond was replaced with the 1,5-disubstituted tetrazole ring (406). A novel procedure for assaying conformational mimicry showed that approximately 88% of the conformations accessible to the cis isomer of the parent dipeptide were also available to the tetrazole analog (406). This index of conformational mimicry represents the percentage of conformations available to the parent peptide which the analog is capable of adopting as measured by the ability to orient the peptide chain and side chains, on either side of the modification in a similar manner to that of the parent peptide (406, 407).

During these studies, the tetrazole analog was found to have more conformational freedom than the cis amide model. This result is explained by the increased valence angle between the C^{α}–C=N of tetrazole analog corresponding to the C^{α}–C=O angle of the cis amide. However, the increase in steric bulk in the tetrazole analog where the amide hydrogen is replaced by nitrogen can cause some constraints. Thus, the conformational flexibility is dependent on the relative importance of these two opposing effects (406). Since another conformational study using Cbz-Proψ[CN_4]Ala-OBzl has

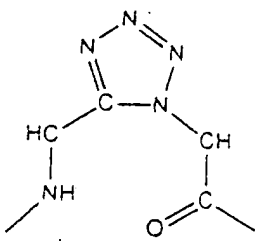


Fig. 20.38 Structure of cyclotetrazole.

also provided similar results (407). the tetrazole ring can be a conformational mimic for the cis amide bond.

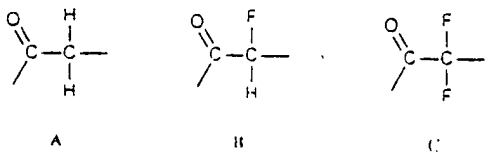
Since proline and N-alkylated amino acids can adopt a cis configuration at their preceding amide bonds in bioactive peptides to bind and recognize their receptors, the tetrazole modified analog can be a useful probe to understand the receptor bound conformation of these peptides. This modification does not contain an adjacent hydrogen-bond donor and acceptor of the cis amide and steric bulk could prevent binding to the receptor. Consequently, activities of tetrazole containing analogs can be strong evidence of the role of a cis amide in receptor recognition while lack of activity will not exclude the cis amide from this consideration.

This modification has been incorporated as a replacement of the Phe-(NMe)Ala bond of a cyclic somatostatin hexapeptide analog to produce c[D-Trp-Lys-Val-Pheψ (CN_4)Ala-Thr] (408). From the conformational studies of this type of somatostatin analogs, it has been suggested that a cis configuration of this bond is required for recognition at the receptors. Since the resulting analog has shown nearly equivalent activity compared to the parent analog, the tetrazole modification functions as a conformational mimic of the cis amide bond in this system. This modification has also been incorporated into the Leu^{8}-Gly^{9} amide bond of deaminooxtocin (409), the Pro^{2}-Pro^{3} and Ser^{6}-Pro^{7} amide bonds of bradykinin (410, 411) and the scissile Phe-Pro bond of HIV protease substrate (412). The bioactivities of these analogs were greatly diminished. If the tetrazole does not grossly distort the topology of the peptide, these results indicate that the cis conformer of the amide bond may not be biologically relevant; or the increased steric hindrance generated from the tetrazole ring and the lack of a hydrogen bond donor, as compared with the cis amide bond, precluded binding of these analogs to the receptors and enzymes.

5.9 Ketomethylene [$C(O)-CH_2$] and Fluoroketomethylene [C(O)-CFR, R=H or F]

The ketomethylene (Fig. 20.39) unit is observed in the structure of the naturally occurring aminopeptidase inhibitor called arphamenines [Argψ($C(O)-CH_2$)Phe-OH] and Argψ[C(O)-CH_2] Tyr-OH (413–415). This modification is conformationally different from the amide bond because the bond between the carbonyl carbon and methylene carbon does not possess any double-bond character. Furthermore, the hydrogens of the methylene cannot be donated to form a hydrogen bond. When ketomethylene replaced the amide bond (416, 417) between Phe and Gly of the angiotensin converting enzyme inhibitor, Bz-Phe-Gly-Pro-OH, the resulting analog is a hundred times more active than the parent peptide (418). This modification has also been incorporated into inhibitors of pepsin (419), aminopeptidase (420), renin (326), and porcine pancreatic elastase (PPE) (421). The mechanism of inhibition for serine protease (i.e., PPE) most likely involves interaction between a serine residue of the enzyme and the ketone carbonyl group of the inhibitor to form a hemiketal structure which resembles the tetrahedral intermediate involved in peptide bond hydrolysis. The replacement of the Met^{28}-Gly^{29} bond of C-terminal octapeptide of cholecystokinin (321) and the Phe^{8}–Gly^{9} bond of the C-terminal hexapeptide of substance P (422, 423) with ketomethylene produced analogs which retained the activity of their respective parent analogs.

The increased potency of fluorinated



A B C

Fig. 20.39 Structures of A, ketomethylene; B, ketofluoromethylene; and C, ketodifluoromethylene.

enzyme inhibitors led to the replacement of the hydrogen(s) of ketomethylene with fluorine (424, 425). The readily hydrated fluoroketone is proposed to mimic the tetrahedral intermediate that forms during the enzyme-catalyzed hydrolysis of a peptide bond. Thus, the ketofluoromethylenes (Fig. 20.39) were considered as a steric replacement of the amide bond. Since the atomic radius of fluorine is similar to that of hydrogen, the steric hindrance of this modification can be minimized. In addition, fluorination can increase the aqueous solubility of the resulting analogs, and with this result that incorporation of these modifications into enzyme inhibitors is now in progress.

5.10 Miscellaneous Amide Isosteres

In addition to the representative amide isosteres described above, many others have been incorporated into bioactive peptides and studied by spectroscopic and computational techniques. Since the planar amide bond in the enzyme substrates must be transformed to a tetrahedral species in the transition state of peptide bond cleavage, various mimics of such intermediates have generated highly active enzyme inhibitors (426). These studies have been extensively reviewed by Rich (427) and Greenlee (428) among others (429, 430). These mimics include methyleneamine, ketomethylene (431) (described in the previous section), phosphonate, phosphonaminate (426, 432), hydroxyethylene (433–435), and dihydroxyethylene (326, 436, 437). Currently, these modifications are widely used for the inhibitors of renin (326, 438, 439), HIV-protease (436, 440–444) and other enzymes (420, 431). Other structures such as epoxides (426), cis-olefins (333), methylene sulfones (323, 326), methylene hydroxyamines (326) and

Table 20.6 Physicochemical Properties of Amide Isosteres[a]

Compound	d (Å)[b]	α (degree)[c]	β (degree)[d]	v (Å^3)[e]
CH_3–C(O)–NH–CH_3	3.8	119	120	69.3
CH_3–NH–C(O)–CH_3	3.8	119	120	69.3
CH_3–CH_2–O–CH_3	3.7	107	113	67.0
CH_3–C(O)–O–CH_3	3.7	116	113	65.8
CH_3–CH_2–NH–CH_3	3.8	111	115	71.6
CH_3–C(O)–CH_2–CH_3	3.9	118	110	75.6
trans-CH_3–CH=CH–CH_3	3.9	122	122	68.2
cis-CH_3–CH=CH–CH_3	3.0	125	236	68.5
CH_3–CH_2–CH_2–CH_3	3.9	111	112	76.0
CH_3–CH(OH)–CH_2–CH_3	4.0	111	113	83.3
CH_3–CH_2–S–CH_3	4.2	110	98	76.2
CH_3–C(S)–NH–CH_3	3.8	115	116	82.0
CH_3–C(O)–N(CH_3)–CH_3	3.9	119	119	85.5

[a] The estimation was carried out using the *N*-methylacetamide [CH_3–C(O)–N(CH_3)–CH_3] and its analogs in which the amide [–C(O)–NH–] of *N*-methylacetamide was replaced with amide isosteres as model compounds. Each model was built to mimic the *trans* form of the amide.

[b] The d denotes the distance between the two methyl groups.

[c] The α denotes the angles for CH_3–C(O)–NH of *N*-methylacetamide and counterparts of the other model compounds.

[d] The β denotes the angles for C(O)–NH–CH_3 of *N*-methylacetamide and counterparts of the other model compounds.

[e] The v denotes the volume of the space occupied by the entire model molecules.

sulfone amides (445, 446) have also been reported as amide isosteres.

Rees et al. systematically quantified some of the amide isosteres using N-methylaceamide and its derivatives (Table 20.6) (333). These steric factors were calculated with the molecular modeling package SYBYL in terms of distance (d) between the two methyl groups, angles for CH_3^C–NH (α) and C–NH–CH_3 (β) and the volume of the space occupied by the entire molecule (v).

6 NONPEPTIDE LIGANDS FOR PEPTINERGIC RECEPTORS

In the previous sections, the considerable progress in obtaining peptide analogs with improved pharmacological properties using peptidomimetics have been described. These peptidomimetic modifications of native peptides have generated many important therapeutic agents. Another approach to peptidomimetics, which could be one of the most important, involves the transformation of peptide structures into nonpeptide structures while retaining the bioactivities at the peptinergic receptors (447). In the opioid area, numerous nonpeptide structures have been devised on the basis of morphine which binds to the same receptors of peptide opioids (448). These efforts have led to many useful analgesics. Portoghese et al. used the message-address concept (449, 450) developed in the peptide area to modify morphine structures (451, 452). The resulting analogs have shown antagonistic activities with high selectivities and potencies.

There has been rational design of nonpeptide analogs based on the structure of native peptides. One of the earliest studies was carried out on the angiotensin converting enzyme inhibitors. Wyvratt and Patchett have reviewed this process (429). Other peptides (CCK, LH-RH, etc.) have also been transformed to nonpeptidic structures. The results are well-described in

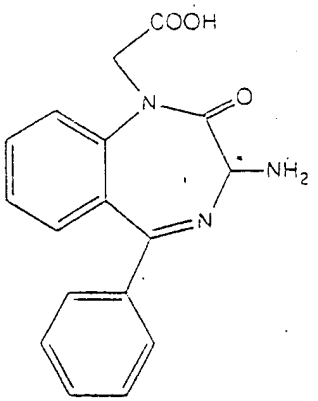
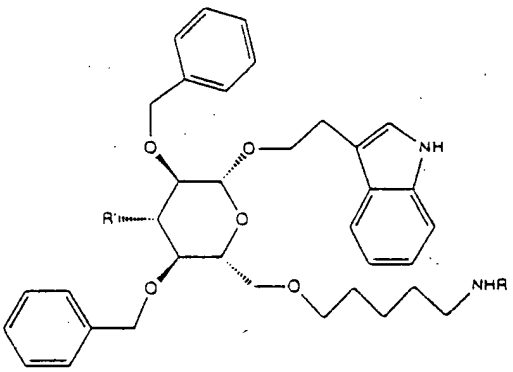


Fig. 20.40 A derivative of bezodiazepine.

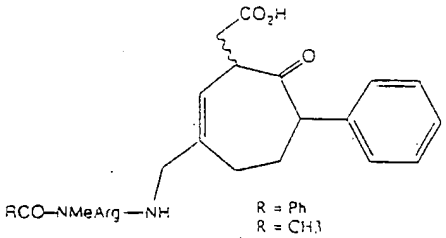
Freidinger's review (453) and other reports (454, 455). Interestingly, 1,4-benzodiazepine and its derivatives (Fig. 20.40) (456) were often used to generate useful therapeutic agents or promising candidates in various categories of peptides showing agonistic and antagonistic activities (453).

Recently, Hirschmann et al. used β-D-glucose as a spacer to arrange the pharmacophoric groups of somatostatin (Fig. 20.41) (457). These nonpeptide analogs are characterized by the rearrangement of the pharmacophores of the corresponding peptides on a glucose scaffold. The resulting analogs show somatostatin activities.

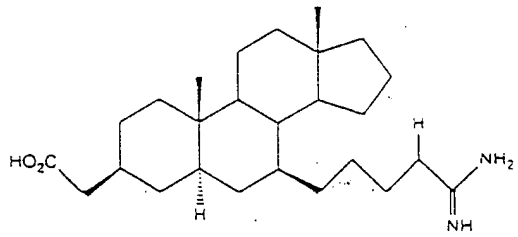


II R = H, R' = OBn
III R = R' = H
IV R = Ac, R' = OBn

Fig. 20.41 A somatostatin analog in which a β-D-glucose is used as a scaffold for the array of pharmacophoric groups.



A



B

Fig. 20.42 RGD peptide analogs in which A, a γ-turn mimic is incorporated; and B, a steroid structure is used as a scaffold to arrange side chains of Arg and Asp.

Because of the development of molecular modeling techniques, the importance of conformational features for such designs have been emphasized. As mentioned, a nonpeptide mimic of turn structure (Fig. 20.32B) was used by Callahan et al. as a spacer for the pharmacophores of RGD peptides providing highly active analogs (Fig. 20.42) (281). Recently, Hirschmann et al, used a steroid as a conformationally restricted spacer leading to an analog with substantial activity (Fig. 20.42B) (458).

7 CONCLUSIONS

In this chapter, various modifications of general peptide structures have been described. The intention has not been to cover chemical methods for each modification and bioactivity profiles completely. However, the general features, conformational preferences and representative applications for many peptidomimetics have been discussed.

Some peptidomimetics are designed as place holders whereas others are isosteric replacements. The effects of these mimetic structures on bioactivity are not generalized for all bioactive peptides. There are cases where a modification in one system leads to bioactive analogs while the same peptidomimetic structure in another peptide family results in inactive analogs. The bioactivities of the resulting analogs vary according to the position of modification. In addition, since each peptidomimetic has unique conformational effects and physicochemical properties, the bioactivities of modified analogs depend on modifications which are incorporated.

The varied bioactivity profiles caused by incorporation of constrained peptidomimetics have been useful in the establishment of conformation-bioactivity relationships of various peptide families. Using such approaches, some laboratories have proposed conformations responsible for the bioactivities and selectivities of many bioactive peptides such as opioids, somatostatins and taste ligands, among others. In addition, these constrained peptidomimetics can be used for the design of peptinergic receptor ligands with higher affinity and structural resemblance.

Other pharmacological properties including metabolic and chemical stability, oral bioavailability and solubility have also been obtained as a result of the introduction of peptidomimetics. For example, incorporation of amide isosteres and other modifications have led to analogs with longer half-lives in biological systems.

It is clear that modified peptides and nonpeptidic analogs represent the present and future in drug design. Hopefully, this chapter has provided insight into the chemistry currently used to explore and design novel drug structures. From such chemistry, new and useful therapeutic agents will emerge.

References

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